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(54) Title: A METHOD OF IMPROVING THE PRODUCTION OF BIOMASS OR A DESIRED PRODUCT FROM A CELL (57) Abstract The production of biomass or a desired product from a cell can be improved by inducing conversion of ATP to ADP without primary effects on other cellular metabolites or functions which is achieved by expressing an uncoupled ATPase activity in said cell and incubating the cell with a suitable substrate to produce said biomass or product. This is conveniently done by expressing in said cell the soluble part (F ₁) of the membrane bound (F ₀ F ₁ type) H ⁺ -ATPase or a portion of F ₁ exhibiting ATPase activity. The organism from which the F ₁ ATPase or portions thereof is derived, or in which the F ₁ ATPase or portions thereof is expressed, may be selected from prokaryotes and eukaryotes. In particular the DNA encoding F ₁ or a portion thereof may be derived from bacteria and eukaryotic microorganisms such as yeasts, other fungi and cell lines of higher organisms and be selected from the group consisting of the gene encoding the F ₁ subunit β or a portion thereof and various combinations of said gene or portion with the genes encoding the other F ₁ subunits or portions thereof. The method can be used i.a. for optimizing the formation of biomass or a desired product by a cell by expressing different levels of uncoupled ATPase activity in the cell, incubating the cell on a suitable substrate, measuring the conversion rate of substrate into biomass or the desired product at each level of ATPase expression, and choosing a level of ATPase expression at which the conversion rate is optimized.		

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A method of improving the production of biomass or a desired product from a cell

This invention relates to a method of improving the production of biomass or a desired product from a cell by inducing conversion of ATP to ADP without primary effects on other cellular metabolites or functions. The invention also relates to a method of optimizing the production of biomass or a desired product from a cell utilizing this first method. The desired product may for example be lactic acid produced by lactic acid bacteria and ethanol or carbondioxide produced by yeast.

BACKGROUND OF THE INVENTION

A wide range of microorganisms are used for the production of various organic compounds and heterologous proteins. One example hereof is the production of lactic acid and other organic compounds by the lactic acid group of bacteria, which results in the acidification and flavouring of dairy products, better known as cheese and yougurt production.

From the microorganism's point of view, the organic compounds which are excreted from the cells are often merely the by-product of a process that is vital to the cells: the production of various forms of free energy (ATP, NAD(P)H, membrane potential, etc.). Therefore, although many of the microorganisms which are being employed in these processes are reasonably well suited for the purpose, there is still a great potential for optimizing the productivity of these organisms when looking from the bioreactor point of vue. Likewise, the production of heterologous proteins by a microorganism is not what the organism was adapted for and also here there is a potential for optimization.

Often when microorganisms are engineered for the purpose of optimizing an industrial production process, the reactions leading to the desired product will affect the delicate balance of co-factors involved in the energy metabolism of the cell. For instance if the glycolytic reactions producing lactate from sugar were somehow to be enhanced (e.g. by overexpressing the glycolytic enzymes) this would automatically lead to the conversion of ADP to ATP. The ratio between the concentrations of ATP and ADP is usually quite high in the growing cell ($[ATP]/[ADP] > 10$), and when the ratio $[ATP]/[ADP]$ changes, the sum of $[ATP]$ and $[ADP]$ still remains virtually constant. Therefore, if in the example above, the enhanced production of ATP changes the $[ATP]/[ADP]$ ratio from 10 to say 30, this will only marginally affect the concentration of ATP. The ADP concentration however will change by a factor of three. The cells will then hardly feel the surplus of ATP but the ADP pool in the cells may be depleted to such an extent that reactions in which ADP is a co-factor or allosteric regulator will be suppressed by the lack of ADP. The result may be that the total flux through the pathway (here through glycolysis) is only marginally increased. In the future, this situation is likely to occur more frequently, as the productivity of bioreactors are optimized by other means, and in these cases, it will be even more important (compared to the normal cell) to regenerate the ADP from ATP, in order to further increase the productivity.

Previously, attempts have been made to decrease the intracellular ATP concentration in yeast, employing sets of reactions which together form futile cycles, see EP patent No. 245 481. Often, the first reaction of a futile cycle is part of the regular metabolic network of the cell, for instance the phosphorylation of a glycolytic intermediate, coupled to the utilisation of ATP. The second reaction, which may also sometimes be part of the

metabolic network, then de-phosphorylates the glycolytic intermediate without regenerating the ATP that was consumed in the first process, the overall effect being that a high energy phosphate bond is consumed. The limited
5 success that this strategy has had so far, is probably due to the fact that it is impossible to obtain a significant futile flux without decreasing the concentration of the phosphorylated intermediate, thereby disturbing the cellular function and ultimately the growth. In addition,
10 when the approach is to decrease the concentration of a glycolytic intermediate, this will effectively remove the substrate for the remaining part of the glycolysis, which will often result in a decreased flux through this pathway, rather than the desired increased flux.

15 Other strategies have been to use chemicals such as dinitrophosphate to stimulate the activity of the plasma membrane H^+ -ATPase by the addition of uncouplers of the membrane potential, or to genetically express the enzyme
20 acid phosphatase in the cytoplasm, an enzyme that will remove phosphate groups from organic metabolites and proteins. However, both of these approaches suffer from the same inherent problem: they are unspecific and a range of cellular reactions/concentrations may be affected. For
25 instance, the acid phosphatase will remove phosphate groups from essential metabolites and proteins, thus disturbing various metabolic fluxes and metabolic regulation. The uncoupling of the plasma membrane H^+ -ATPase will disturb the intracellular pH in addition to the gradient of numerous ions across the cytoplasmic membrane.
30 Besides, the addition of chemicals such as dinitrophosphate is undesirable for most purposes.

SUMMARY OF THE INVENTION

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The idea of the invention is to use a highly specific and clean way to increase the intracellular level of ADP,

which does not suffer from the limitations described above: to express in a well-controlled manner an enzyme that has ATP-hydrolytic activity in the living cell without producing other products and without coupling this activity to energy conservation. Such an enzymatic activity is of course not likely to be found in a normal cell, because the cell would then lose some of its vital energy reservoir.

Accordingly the present invention provides a method of improving the production of biomass or a desired product from a cell, the method being characterized by expressing an uncoupled ATPase activity in said cell to induce conversion of ATP to ADP without primary effects on other cellular metabolites or functions, and incubating the cell with a suitable substrate to produce said biomass or product.

One of the normal enzymes that comes closest to the ideal ATP-hydrolyzing enzyme, is the membrane bound H^+ -ATPase. This huge enzyme complex consists of two parts, the membrane integral part (F_0) and the cytoplasmic part (F_1). Together the two parts couples the hydrolysis of ATP to ADP and inorganic phosphate (P_i), to translocation of protons across the cytoplasmic membrane, or vice versa, using the proton gradient to drive ATP synthesis from ADP and P_i .

The method of the invention is conveniently carried out by expressing in said cell the soluble part (F_1) of the membrane bound (F_0F_1 type) H^+ -ATPase or a portion of the F_1 exhibiting ATPase activity.

The membrane bound H^+ -ATPase complex is found in similar form in prokaryotic as well as eukaryotic organisms, and thus F_1 and portions thereof expressing ATPase activity

can be expressed in both prokaryotic and eukaryotic cells.

5 The organism from which the F₁ ATPase or portions thereof is derived, or in which the F₁ ATPase or portions thereof is expressed, may be selected from prokaryotes and eukaryotes, in particular from bacteria and eukaryotic microorganisms such as yeasts, other fungi and cell lines of higher organisms, in particular bakers and brewers
10 yeast.

A particularly interesting group of prokaryotes to which the method according to the invention can be implemented, i.a. in the dairy industry, are lactic acid bacteria of
15 the genera *Lactococcus*, *Streptococcus*, *Enterococcus*, *Lactobacillus* and *Leuconostoc*, in particular strains of the species *Lactococcus lactis* and *Streptococcus thermophilus*. Other interesting prokaryotes are bacteria belonging to the genera *Escherichia*, *Zymomonas*, *Bacillus* and *Pseudomonas*, in particular the species *Escherichia coli*, *Zy-*
20 *momonas mobilis*, *Bacillus subtilis* and *Pseudomonas putida*.

In an expedient manner of carrying out the method according to the invention the cell is transformed or trans-
25 fected with an expression vector including DNA encoding F₁ or a portion thereof exhibiting ATPase activity under the control of a promoter functioning in said cell, and said DNA is expressed in the cell. Said DNA encoding F₁
30 or a portion thereof may be derived from a prokaryotic or a eukaryotic organism, and it may be either homologous or heterologous to said cell.

The F₁ part of the bacterial H⁺-ATPase complex consists
35 of several subunits that together are responsible for catalyzing ATP hydrolysis: the β -subunit is thought to carry the actual hydrolytic site for ATP hydrolysis, but

in vitro ATPase activity requires that the β -subunit forms a complex together with the α - and γ -subunit ($\alpha_3\gamma\beta_3$). The activity of this complex is modulated by the ϵ -subunit, so that the *in vitro* activity of the $\alpha_3\gamma\beta_3\epsilon$ complex is five fold less than the $\alpha_3\gamma\beta_3$ complex.

In a specific embodiment of the method according to the invention said DNA encoding F_1 or a catalytic active portion thereof, is derived from *Escherichia coli*, *Streptococcus thermophilus* or *Lactococcus lactis* and is selected from the group consisting of the gene encoding the F_1 subunit β or a catalytically active portion thereof and various combinations of said gene or portion with the genes encoding the F_1 subunits δ , α , γ and ϵ or catalytically active portions thereof.

In particular said DNA encoding F_1 or a portion thereof may be selected from the group consisting of the *Escherichia coli*, *Streptococcus thermophilus* and *Lactococcus lactis* genes *atpHAGDC* (coding for subunits δ , α , γ , β , ϵ), *atpAGDC* (coding for subunits α , γ , β , ϵ), *atpAGD* (coding for subunits α , γ , β), *atpDC* (coding for subunits β , ϵ) and *atpD* (coding for subunit β alone).

Particularly interesting eukaryotes are the yeasts *Saccharomyces cerevisiae*, *Phaffia rhodozyma* or *Trichoderma reesei*, and the DNA encoding F_1 or a portion thereof may be derived from such organisms and is selected from the group consisting of the gene encoding the F_1 subunit β or a portion thereof and various combinations of said gene or portion with the genes encoding the other F_1 subunits or portions thereof.

Vectors including DNA encoding the soluble part (F_1) of the membrane bound (F_0F_1 type) H^+ -ATPase or a portion of F_1 exhibiting ATPase activity, derived from the lactic acid bacteria *Lactococcus lactis* and *Streptococcus ther-*

mophilus and from the yeasts *Saccharomyces cerevisiae*, *Phaffia rhodozyma* or *Trichoderma reesei* are also comprised by the invention as well as expression vectors including such DNA under the control of a promoter capable
5 of directing the expression of said DNA in a prokaryotic or eukaryotic cell.

Specific vectors according to the invention are plasmids including DNA encoding the soluble part (F₁) of the membrane bound (F₀F₁ type) H⁺-ATPase or a portion of F₁ exhibiting ATPase activity, said DNA being derived from
10 *Lactococcus lactis* subsp. *cremoris* (SEQ ID No. 1), *Lactococcus lactis* subsp. *lactis* (SEQ ID No. 6), *Streptococcus thermophilus* (SEQ ID No. 10), *Phaffia rhodozyma* (SEQ ID
15 No. 14), and *Trichoderma reesei* (SEQ ID No. 16).

Further, the invention provides a method of optimizing the formation of biomass or a desired product by a cell, the method being characterized by expressing different
20 levels of uncoupled ATPase activity in the cell, incubating the cell on a suitable substrate, measuring the conversion rate of substrate into biomass or the desired product at each level of ATPase expression, and choosing a level of ATPase expression at which the conversion rate
25 is optimized.

Often, but not always, the optimization of a given product flux produced by a cell will entail the attainment of either maximum or minimum conversion rate of a substrate.

30

In an expedient manner of practicing this method of the invention a number of specimens of said cell are transformed or transfected with their respective expression vector each including DNA encoding a different portion of
35 the cytoplasmic part (F₁) of the membrane bound (F₀F₁ type) H⁺-ATPase up to and including the entire F₁, each portion exhibiting ATPase activity, said DNA in each ex-

pression vector being under the control of a promoter functioning in said cell, incubating each cell specimen on a suitable substrate, measuring the conversion rate of substrate into biomass or the desired product in each specimen, and choosing a specimen yielding an optimal conversion rate. In a particular embodiment of this manner, which is especially suited for scientific studies, the promoter in each expression vector is an inducible promoter, and each cell specimen is grown at different concentrations of inducer in order to fine-tune the optimal conversion rate.

In a preferred manner of practicing the above method of optimizing the performance of a cell a number of specimens of said cell are transformed or transfected with their respective expression vector including DNA encoding a portion of the cytoplasmic part (F₁) of the membrane bound (F₀F₁ type) H⁺-ATPase up to and including the entire F₁, said portion exhibiting ATPase activity, said DNA in the respective expression vectors being under the control of each of a series of promoters covering a broad range of promoter activities and functioning in said cell, incubating each cell specimen on a suitable substrate, measuring the conversion rate of substrate into biomass or the desired product by each specimen, and choosing a specimen yielding an optimal conversion rate. In a more preferred embodiment of this manner, which is well suited to establish an optimal production strain, the respective expression vectors include DNA encoding different such portions of F₁ up to and including the entire F₁, each DNA in respective expression vectors being under the control of each of a series of promoters covering a broad range of promoter activities and functioning in said cell.

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Also in this method of the invention the DNA encoding a portion of F₁ up to and including the entire F₁ may be

derived from a prokaryotic or a eukaryotic organism, and it may be either homologous or heterologous to said organism. The specific DNAs mentioned above may also conveniently be employed in this method.

5

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1. A linear representation of the plasmids constructed for modulating the cellular [ATP]/[ADP] ratio in *E. coli* (not drawn to scale).

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Figure 2. Effect of induction of F₁-ATPase activity on the growth of *E. coli* in batch culture. Cells were grown for more than 10 generations in minimal medium supplemented with glucose (0.4 g/l), ampicillin (0.1 g/l) and the indicated concentration of inducer, IPTG.

15

Figure 3. Effect of ATPase expression on the intracellular concentration of ATP and ADP (concentration in arbitrary units), and on the ratio [ATP]/[ADP].

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Figure 4 Effect of increased ATPase expression on the glycolytic flux.

25 DETAILED DESCRIPTION OF THE INVENTION

Many biosynthetic reactions in the living cell (anabolism), require an input of free energy (ATP), which is generated through a series of degrading reactions (catabolism). In the aerobic cell, there are two routes for ATP synthesis: 1) substrate level phosphorylation, where an energy rich phosphoryl group is transferred directly from a high energy intermediate metabolite to ADP, and 2) oxidative phosphorylation, where the free energy is first transformed into redox free energy by oxidizing the energy source, then into a proton gradient by respiration and finally the proton gradient is used by the H⁺-ATPase

30

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to drive ATP synthesis from ADP and inorganic phosphate. In other cases, e.g. anaerobic growth, there is only the first route, substrate level phosphorylation, that can be used for ATP synthesis. An example hereof is the homolac-
5 tic LAB, where lactose is converted through the glycolytic pathway to lactic acid, which is excreted from the cells and thereby lowers the pH of the growth medium (usually milk products). With respect to ATP generation, homolactic fermentation is a very inefficient process,
10 and only four moles of ATP are produced from 1 mole of lactose through substrate level phosphorylation.

The anabolic (ATP consuming) and catabolic (ATP producing) fluxes are normally well balanced in the living
15 cell, and therefore, in the wild-type cell under normal growth conditions, the catabolic fluxes will be proportional to the anabolic fluxes. If a reaction is introduced that for instance hydrolyzes ATP in the cell and thereby lowers the cellular energy state (i.e. the
20 $[ATP]/[ADP]$ ratio), then either catabolism should increase or anabolism (growth) should decrease in order to make the consumption rate equal the production rate again. Which of these two scenarios will take place depends on whether, initially, the growth rate of the cell
25 is limited through anabolism or through catabolism, i.e. whether there is a surplus or a shortage of energy in the cell to begin with. If there is a shortage of energy, then the rate of the anabolic reactions is limited by catabolism and these reactions will be sensitive to changes
30 in the cellular energy state. Introduction of an ATP-hydrolyzing reaction is then most likely to affect the growth rate of the cells. On the other hand, if there is a surplus of energy, then the growth rate will be limited mainly by the anabolic reactions; the rate of anabolism
35 will be insensitive to a decrease in the energy state, but the catabolic rate may increase due to a decrease in product inhibition at lower $[ATP]/[ADP]$ ratio.

In vitro, the F₁ part of the H⁺-ATPase complex has been shown to have ATPase activity, see above. But so far nobody has managed to use the F₁ complex to stimulate the glycolytic flux, or even to show that the F₁ complex can hydrolyze ATP in intact cells. Indeed, when we first tried to overexpress the F₁ complex, consisting of the genes for the subunits α , γ , β and ϵ , this had virtually no effect on the growth of *E. coli*, even when the genes were transcribed from the maximally induced *tac* promoter and on a very high copy number vector (derived from pUC18). One skilled in the art of gene expression in *E. coli* will appreciate that this combination is one of the most efficient expression systems that exists for this organism.

We then decided to try to express different combinations of subunits of the F₁ complex, in order to see if other combinations of subunits would be more powerful. Plasmids were constructed containing various combinations of the genes encoding the F₁ part of the bacterial F₁F₀-ATPase complex from *E. coli*. The genes were expressed, either from an inducible (*lac*-type) promoter at various concentrations of inducer or from a series of constitutive promoters of varying promoter activity. These plasmids should express various levels of ATPase activity when introduced into the bacterial cell. Depending on which F₁ genes are present on the plasmid and the strength of the promoter which is used to drive the expression, we observed various degrees of inhibition of the growth of the cells harbouring these plasmids. Surprisingly, the beta subunit alone and in combination with the epsilon subunit turned out to be far more active *in vivo* than the entire F₁ complex.

The objective of this work was to affect the energy state of the cells, as reflected in the ratio [ATP]/[ADP]. We therefore measured the intracellular concentration of ATP

and ADP in growing cells expressing various activities of F₁-ATPase. Indeed the ATP concentration decreased slightly with increasing ATPase activity and the ADP concentration increased, and therefore the [ATP]/[ADP] ratio
5 decreased (the effect on the ATP concentration was less than the effect on the ADP concentration as expected, see above). We also calculated the glycolytic flux through the cells with various levels of ATPase activity. We found that the flux through the glycolytic pathway was
10 first stimulated with increasing expression of ATPase activity, until a certain (optimal) ATPase activity which gave maximal glycolytic flux. Further increase of ATPase expression resulted in a lower glycolytic flux, due to a secondary effect of the ATPase activity on the growth of
15 the cells. This emphasizes the need for optimization of gene expression rather than merely overexpressing the genes.

EXAMPLE 1

20

ATP hydrolysis and enhanced glycolytic flux in *Escherichia coli*, using an inducible promoter

25 Restriction enzymes, T4 DNA polymerase, calf intestine phosphatase (CIP) were obtained from Pharmacia.

Procedures for DNA isolation, cutting with restriction enzymes, filling in sticky DNA ends with T4 DNA polymerase in the presence of dATP, dCTP, dGTP and dTTP,
30 treatment with calf intestine phosphatase to remove phosphate groups from 5' DNA ends and ligation of DNA fragments are carried out by standard methods as described by Maniatis et al., 1982.

Extraction and measurement of ATP and ADP

0.9 ml of cell culture was mixed with 0.9 ml of (80 °C) phenol (equilibrated with 10 mM Tris, 1 mM EDTA pH=8) and immediately vortexed vigorously for 10 seconds. After 1 hour at room temperature the sample was vortexed again for 10 seconds and the two phases were separated by centrifugation at 14000 rpm for 15 minutes, and then residual phenol in the water phase was removed by extraction with 1 volume of chloroform. ATP and ADP concentrations were then measured, using a luciferin-luciferase ATP monitoring kit (obtained from and used as recommended by LKB, except that 3 mM of phosphoenol-pyruvate was added). [ATP] was measured first. Subsequently the ADP in the same sample was converted to ATP by adding pyruvate kinase, and [ADP] was recorded as the concomitant increase in luminescence.

Construction of plasmids carrying combinations of the *E. coli atp* genes

The following combinations of *E. coli* genes coding for F₁ subunits were chosen for expressing ATPase activity in *E. coli*: 1. *atpAGDC* (subunits α , γ , β , ϵ), 2. *atpAGD* (subunits α , γ , β), 3. *atpDC* (subunits β , ϵ), and 4. *atpD* (subunit β alone).

Cloning of fragments carrying *atp* genes onto pUC19

The plasmid pBJC917 (von Meyenburg, K., et al., 1984) which carries the entire *atp* operon was cut with

1) the restriction enzyme *DraIII*, and a 5009 bp DNA fragment containing the *atpAGDC* genes was isolated;

2) the restriction enzymes *DraIII* and *Tth111I*, and a 4106 bp DNA fragment containing the *atpAGD* genes was isolated;

3) the restriction enzymes *DraIII* and *SacII*, and a 2364 bp DNA fragment containing the *atpDC* genes was isolated;

4) the restriction enzymes *AvaI* and *Tth111I*, and a 1472 bp DNA fragment containing the *atpD* gene was isolated.

In all four cases the fragments were then treated with T4 DNA polymerase to create blunt ends, and subsequently the fragments were ligated into the cloning vector pUC19 (Yanisch-Perron et al., 1985) which had been cut with *SmaI* and treated with CIP.

The four ligation mixtures were transformed into the *E. coli* strain JM105 (Yanisch-Perron et al., 1985), and the transformation mixtures were plated on LB (Luria-Bertani broth; Maniatis et al., 1982) plates containing 100 µg/ml ampicillin and 75 µg/ml 5-bromo-4-chloro-3-indolyl-β-D-galactoside (X-gal). In this strain background (JM105), plasmids formed by religation of pUC19 will give blue colonies, whereas plasmids that carry foreign DNA fragments inserted into the *SmaI* site of pUC19, will give white colonies. A number of white colonies from the four transformations were therefore picked for further analysis: plasmid DNA was isolated and analysed by cutting with various restriction enzymes. Clones were identified from each of the four series which had the desired fragment inserted into the *SmaI* site of pUC19, and in the proper orientation. These four plasmids were named, respectively: pATP-AGDC, pATP-AGD, pATP-DC and pATP-D, with reference to the specific *atp* genes carried by the plasmid.

Cloning combinations of the *atp* genes under the control of an inducible (*tac*) promoter

In order to be able to control the expression of the ATPase activity, we selected the expression vector pTTQ18

(Starck, 1987). This vector is a derivative of pUC18 (Yanisch-Perron *et al.*, 1985), which carries a *tac* promoter and the lactose repressor gene, *lacI*. Immediately downstream of the *tac* promoter is a multiple cloning site (MCS; the polylinker from pUC18) in which genes can be inserted to be expressed from the *tac* promoter. The *tac* promoter is of the *lac*-type, i.e. repressed by the lactose repressor and inducible with isopropyl- β -D-thiogalactoside (IPTG).

10

The four plasmids, pATP-AGDC, pATP-AGD, pATP-DC and pATP-D were cut with *KpnI* and *XbaI*, which gave the four DNA fragments, 5023, 4120, 2378 and 1486 respectively. After purification, the fragments were ligated into the cloning vector, pTTQ18, which had also been cut with *KpnI* and *XbaI* (see figure 1). The ligation mixtures were transformed into *E. coli* K-12 MC1000 (Casabadan and Cohen, 1980), and the transformation mixtures were plated on LB plates containing 100 μ g/ml ampicillin. A number of colonies from the four transformations were therefore picked for further analysis: plasmid DNA was isolated and analysed by cutting with various restriction enzymes. Clones were identified from each of the four series which had the desired fragment inserted into the MCS of pTTQ18 in the proper orientation. These four plasmids were named, respectively: pTAC-AGDC, pTAC-AGD, pTAC-DC and pTAC-D, with reference to the specific *atp* genes carried by these plasmid and the *tac* promoter used for their expression. For the purpose of subsequent physiological studies, the plasmids were transformed into the *E. coli* K-12 strain LM3118, which is used routinely for physiological experiments in this laboratory. The corresponding names for the LM3118 strain carrying these four plasmids are PJ4332, PJ4333, PJ4335 and PJ4334, respectively.

35

Effect of induction of ATPase activity on the growth of *E. coli* on plates

The strains containing the four plasmids were streaked on LB plates containing ampicillin (100 µg/ml) and 1 mM of IPTG which should give maximum expression from the *tac* promoter. Table I shows how the four strains responded: the strain carrying plasmid pATP-AGDC, which contains the genes for the four subunits, α , γ , β and ϵ , was only very slightly affected in growth, even in the presence of 1 mM IPTG. The other three plasmids, pTAC-AGD, pTAC-DC and pTAC-D caused severe growth inhibition in the presence of 1 mM IPTG, where colonies were no longer visible. With intermediate concentrations of IPTG, 0.01 mM and 0.1 mM, the plasmids affected the growth of their host cells to different extents: pTAC-AGD was the most active, giving rise to a strong inhibition of growth already with 0.01 mM IPTG, a concentration which gave only a slight inhibition with the plasmid pTAC-DC and no inhibition of the strain with pTAC-D. With 0.1 mM IPTG, colonies were hardly visible for the strain that carried the pTAC-AGD, the plasmid pTAC-DC caused strong growth inhibition, whereas the effect of pTAC-D was significant but small.

Table I

Strain	Plasmid	- IPTG	0.01 mM IPTG	0.1 mM IPTG	1 mM IPTG
PJ4332	pTAC-AGDC	++++	++++	++++	+++
PJ4333	pTAC-AGD	++++	++	+	-
PJ4335	pTAC-DC	++++	+++	+	-
PJ4334	pTAC-D	++++	++++	++	-

++++ = normal colony size; +++ = slight inhibition; ++ = 1/2 normal size; + = 1/10 normal size; - = no growth

The effect of ATPase expression from the four plasmids above was also studied in the *E. coli* mutant LM3115, in which the entire *atp* operon on the chromosome is deleted, but which grows with almost wild-type growth rate on LB

medium. With this strain transformed with the four plasmids we observed a similar pattern of growth inhibition on LB plates as a function of IPTG concentration. This shows that the effect of ATPase expression was independent of the presence of the normal *atp* operon.

Effect of induction of ATPase activity on the growth of *E. coli* in liquid cultures

The effect of induction of ATPase was also studied with cells grown in liquid cultures. For this purpose we chose the strain PJ4333, carrying the plasmid pTAC-AGD, because this plasmid appears to be the most active with respect to the inhibitory effect on the growth of *E. coli*. Figure 2 shows the growth of PJ4333 in minimal medium supplemented with a limiting concentration of glucose (0.4 g/l) and ampicillin (0.1 g/l), without IPTG and in the presence of increasing concentrations of IPTG. We observed that the growth rate of the strain was practically constant (within some 10%) with increasing amounts of IPTG up to about 30 μ M. At higher than 40 μ M IPTG, the growth of the cells were slightly inhibited, in accordance with the experiments on plates, see above.

However, what was affected was the final density of cells that one obtains from the limited amount of glucose that was included in each culture: The more ATPase that is expressed in the cells, the lower the yield of cell mass. Apparently, the cells become less economic with respect to converting the glucose into biomass, or in other words they consume more glucose per cell synthesized. If this is due to the expression of ATPase activity, then we would expect to see an effect hereof on the energy state of the cells. We therefore measured the concentrations of ATP and ADP in the cells growing with different expression levels of ATPase activity.

Indeed, the intracellular ATP concentration decreased gradually and the ADP concentration increased, with increased expression of ATPase; therefore the [ATP]/[ADP] ratio decreased with increased expression of ATPase, which imply that the increased glucose consumption is the result of increased ATP conversion to ADP, see figure 3. The actual flux of glucose through the cells (J_{gluc} , mmol glucose / g cell dry weight / hour) is also interesting, because this value tells us whether the performance of the cell increased as the ATPase activity increased. J_{gluc} can be calculated from the yield, Y (g cell dry weight / mol glucose) and the specific growth rate of the culture, μ (1/hours):

$$J_{\text{gluc}} = \mu/Y$$

Figure 4 shows how the flux of glucose changed as the activity of ATPase increased: the glycolytic flux increased gradually as the ATPase expression increased, until a maximum was reached (at 30 μM IPTG). Further increase of ATPase expression had a slightly negative effect on the glucose flux. This was probably because the energy state of the cells became so low that this had a negative effect on some anabolic reactions, since the growth rate was lower for the culture that was grown in the presence of 40 μM IPTG.

The expression of subunits of the F_1 part of the bacterial H^+ -ATPase lowers the energy state of the bacterial cell. This is due to hydrolysis of ATP into ADP and P_i . The expression of ATPase activity does not affect the growth rate of *E. coli* much at low levels of expression, but the efficiency by which the substrate is converted into biomass was strongly reduced. Under the set of conditions used here, the expression of ATPase activity has a stimulatory effect on the rate by which the cells consumes the exogenous glucose.

EXAMPLE 2**Expression of F₁-ATPase activity from constitutive promoters in *E. coli***

5 In example 1 we used a *lac*-type promoter system to modulate the expression of the F₁ ATPase subunits in *E. coli*. However, for the optimization of gene expression for instance in industrial bioreactors or for the use in fermented food products, the use of *lac* type promoters is not always feasible. In this example we illustrate the optimization of F₁-ATPase expression in *E. coli*, using a series of constitutive promoters of different strength, to control the expression of the *atpAGD* genes which here originates from *E. coli*. The constitutive promoters (CP promoters) were selected from a library of artificial promoters which had previously been cloned onto a shuttle vector for *E. coli* and *L. lactis*, pAK80 (Israelsen et al., 1995) as described in our co-pending PCT patent application PCT/DK97/00342. The selected plasmid derivatives of pAK80 were pCP34, pCP41 and CP44 (CPX cloning vectors). The *atpAGD* fragment from pTAC-AGD (from example 1) was first subcloned in a polylinker in order to have the *atpAGD* fragment flanked by two *Bam*HI sites. Subsequently, this *Bam*HI fragment was cloned into the unique *Bam*HI site downstream of the CP promoters on the plasmids pCP34, pCP41 and CP44, resulting in the plasmids, pCP34::*atpAGD*, pCP34::2*atpAGD*, pCP41::*atpAGD* and CP44::*atpAGD*, where pCP34::2*atpAGD* contains two *atpAGD* fragments in tandem.

Subsequently, the strains were characterized with respect to growth rate, growth yield and glycolytic flux in glucose minimal medium supplemented with 200 µg/ml erythromycin, essentially as described in example 1, see table 2.

The expression of the F₁-ATPase subunits had a slightly negative effect on the growth rate as the expression level increased. The effect on growth yield was much stronger and at the highest expression level the growth yield had
 5 dropped to 40 % of the initial value. The glycolytic flux was stimulated 70% at the highest expression level of ATPase, and at this expression level the growth rate was lowered by 30%.

Table 2. Effect of expression of uncoupled F₁-ATPase activity (*E. coli* α , γ , β subunits) in *E. coli*

Plasmid	Biomass yield gdw/mmol glucose	Growth rate, μ h ⁻¹	Glucose flux mmol glu- cose/h/gdw	Biomass yield %	Growth rate %	Glucose flux
pCP41	0,067	0,47	6,9	100	100	100
pCP41::atpAGD	0,047	0,42	9,1	69	90	131
pCP34	0,063	0,41	6,6	100	100	100
pCP34::atpAGD	0,034	0,34	9,9	54	81	149
pCP44	0,067	0,44	6,5	100	100	100
pCP44::atpAGD	0,027	0,30	11,2	40	69	172

EXAMPLE 3

Expression of *E. coli* F₁-ATPase activity from constitu-
 5 tive promoters in *L. lactis*.

The plasmids from example 2 which express the *E. coli* F₁-ATPase subunits to various extent are also capable of replicating in *L. lactis*, and could therefore be used to
 10 test whether the *E. coli* F₁-ATPase subunits can be used to hydrolyse ATP in *L. lactis*.

The plasmids pCP34::atpAGD, pCP34::2atpAGD and pCP41::atpAGD, were transformed into the *L. lactis* subspecies *cremoris* strain, MG1363, which is used routinely for physiological experiments in this laboratory. In addition we transformed the respective vectors, pCP34 and pCP41 in order to have proper control strains. Subsequently, the resulting transformants were characterized with respect to growth rate, growth yield and glycolytic flux, in comparison to the respective vectors, pCP34 and pCP41, by growing the various cultures in defined medium (SA medium) supplemented with a limiting concentration of glucose (0.1%), see table 3.

Table 3. Expression of *E. coli* F₁-ATPase in *L. lactis*

Plasmid	Biomass yield gdw/mmol glucose	Growth rate, μ h ⁻¹	Glucose flux mmol glu- cose/h/gdw	Biomass yield %	Growth rate %	Glucose flux %
pCP34	0,073	0,664	9,161	100	100	100
pCP34::atpAGD	0,071	0,653	9,230	97,5	98,3	100,8
pCP34::2atpAGD	0,069	0,655	9,560	94,6	98,7	104,4
pCP41	0,072	0,645	8,925	100	100	100
pCP41::atpAGD	0,070	0,590	8,461	96,5	91,5	94,8

The results show that the plasmids pCP34::atpAGD and pCP34::2atpAGD did affect the growth yield and the glycolytic flux to some extent, but the plasmids were far less efficient in *L. lactis*, compared to *E. coli*. This was probably a consequence of a lower expression of the *E. coli* ATPase subunits, or some of these, in *L. lactis*, due to a lower copy number of the pAK80 vector in *L. lactis* (5-10), and due to differences in the translational efficiency of the three individual *atp* genes which originates from *E. coli*. The plasmid pCP41::atpAGD also resulted in a lower growth yield, indicating that also in

this case uncoupled ATP hydrolysis was taking place. However, the pCP41::atpAGD plasmid had a relatively strong inhibitory effect on the growth rate and therefore the glycolytic flux was not increased by this plasmid. It is possible that the heterologous expression of *E. coli* ATPase subunits resulted in growth inhibition due to effects other than ATP hydrolysis, e.g. by interfering with the function of the *L. lactis* F₁F₀ H⁺-ATPase complex.

10 EXAMPLE 4

Expression of *L. lactis* F₁-ATPase subunits β and ϵ , in *L. lactis*.

15 In the example above we showed that the expression of F₁-ATPase subunits from *E. coli* in *L. lactis*, resulted in only a small stimulation of the glycolytic flux. It is possible that the heterologous expression of *E. coli* ATPase subunits resulted in growth inhibition due to effects other than ATP hydrolysis, e.g. by interfering with the function of the *L. lactis* F₁F₀ H⁺-ATPase complex. In the present example we have expressed the *L. lactis* F₁-ATPase subunits, β and ϵ , in *L. lactis*, as this appeared to be an effective combination of subunits when expressed in *E. coli*, see example 1. The atpDC_{Llc} genes from *L. lactis* subspecies *cremoris* (SEQ ID No. 1) was cloned on a 2.5kb HindIII fragment into the HindIII restriction site on the standard cloning vector, pBluescript, into *E. coli* K-12, strain BOE270. Subsequently, the atpDC_{Llc} genes were cut out on a 2.5kb BamHI-SalI fragment and cloned into 5 expression vectors, pCP32, pCP34, pCP37, pCP41 and pCP44 which had been digested with BamHI and SalI, resulting in the plasmids pCP32::atpDC_{Llc}, pCP34::atpDC_{Llc}, pCP37::atpDC_{Llc}, pCP41::atpDC_{Llc} and pCP44::atpDC_{Llc}, respectively, where the lacLM genes downstream of the CP promoters, have been replaced with the atpDC_{Llc} genes. These plasmids should express the *L. lactis* F₁-ATPase

subunits, β and ϵ , to different extent. The plasmids were then transformed into MG1363 with selection for the erythromycin resistance carried by these vectors. Experiments were then performed to test whether the constructs

5 resulted in conversion of ATP into ADP in *L. lactis*. The strains carrying the different constructs was then grown in GM17 medium supplemented with 5 μ g/ml erythromycin. The plasmids did not have a strong effect on the growth rate of the cultures, which remained close to the growth

10 rate of the respective vector control plasmids. The yield of biomass, however, decreases for all the cultures by up to 17%, which shows that the cultures did indeed express uncoupled ATPase activity, see table 4.

15 Table 4. Effect of expression of *L. lactis* β and ϵ subunits on acid production by *L. lactis*, at 30°C and with initial pH 6.7.

Plasmid	Biomass [*] OD ₄₅₀	Final pH [*]	Acid formation, relative to biomass [*] % of vector
pCP34	5.08	4.27	100
pCP34::atpDC11c	4.72	4.31	98.0
pCP41	4.66	4.34	100
pCP41::atpDC11c	5.21	4.24	113.5
pCP37	4.89	4.28	100
pCP37::atpDC11c	4.63	4.24	116.1
pCP32	4.86	4.34	100
pCP32::atpDC11c	3.95	4.36	116.9

20 Each value is the average of 3-4 independent cultures. The acid production was calculated from the pH change, and normalized by the biomass produced.

The GM17 growth medium used in these experiments contains a surplus of glucose (1%), and growth only stops when the

25 pH of the growth medium becomes lower than approximately pH 4.3. To some extent, this mimics the situation that the lactic acid bacteria experience during cheese and

yougurt production. In this medium, the growth yield, in terms of the final cell mass of the cultures, reflects the acid production by these cultures.

5 In these cultures, the expression of F_1 -ATPase subunits will increase three fold at approximately OD600 equal to 1.5. This is a consequence of the three fold amplification of the plasmid copy number that has been shown to take place at this point of the growth curve. In reality,
10 the effect of expressing the F_1 -ATPase subunits may therefore be larger.

To test this hypothesis, we grew some of the strains which expressed the *L. lactis* F_1 -ATPase subunits β and ϵ
15 in batch cultures of GM17 medium which had been adjusted to pH 5.9, see Table 5. In addition, the temperature of the growth medium may also affect the plasmid copy number and thus the expression of the F_1 -ATPase subunits. The experiments were therefore performed at 37°C.

20

Table 5. Effect of expression of *L. lactis* β and ϵ subunits on acid production by *L. lactis*, at 37°C and with initial pH 5.9.

Plasmid	Biomass ^a OD ₄₅₀	Ffinal pH ^a	Acid formation, relative to biomass ^a % of vector
pCP34	1.24	4.95	100
pCP34::atpDC _{11c}	1.06	4.87	141.4
pCP37	1.00	4.96	100
pCP37::atpDC _{11c}	0.58	4.92	188.4

25

Clearly, the effect of the F_1 -ATPase activity was much stronger under these growth conditions: the amount of acid produced was almost doubled for the strain carrying the plasmid pCP37::atpDC_{11c}.

30

EXAMPLE 5

Expression of the F₁-ATPase subunits, α , γ , and β , from *L. lactis* subspecies *cremoris* in *L. lactis* subspecies *cremoris*.

In example 4, only the *L. lactis* F₁-ATPase β and ϵ subunits were expressed in *L. lactis*. However, from the experiments with *E. coli* (example 1), we know that the simultaneous expression of subunits α , γ , and β , is a more powerful combination, which could also be the case for *L. lactis*. In order to obtain the same strong stimulation of the glycolytic flux and acid production in *L. lactis*, a set of vectors similar to the vectors described in example 4 was constructed, in which the *atpAGD_{Llc}* genes derived from *L. lactis*, encoding the subunits α , γ , and β (SEQ ID No. 1) was expressed from CP promoters with different activities. The *atpAGD_{Llc}* genes from *L. lactis* was cloned on a 2.5 kb *Bam*HI-*Sal*I fragment into the 5 vectors, pCP32, pCP34, pCP37, pCP41 and pCP44, resulting in the plasmids, pCP32::*atpAGD_{Llc}*, pCP34::*atpAGD_{Llc}*, pCP37::*atpAGD_{Llc}*, pCP41::*atpAGD_{Llc}*, pCP44::*atpAGD_{Llc}*, respectively, where the *lacLM* genes downstream of the CP promoters, has been replaced with the *atpAGD_{Llc}* genes. These plasmids will express the *L. lactis* F₁-ATPase subunits α , γ , and β , to different extent. The plasmids were transformed into MG1363 with selection for the Erythromycin resistance carried by these vectors. Experiments were then performed to show that the constructs were effective in ATP hydrolysis in *L. lactis* and to what extent the glycolytic flux was enhanced, by growing the five different constructs in GM17 medium supplemented with erythromycin, and measuring the growth rate, ATP and ADP concentrations, the yield of biomass and the rate of acid production.

EXAMPLE 6

Expression of F₁-ATPase subunits from *L. lactis* subsp *lactis*, in *L. lactis* subspecies *lactis*.

5 In the examples 3-5 above, we used the strain *L. lactis* subsp. *cremoris*, MG1363. This strain is plasmid-free and is used routinely in our laboratory as a simple model organism for our physiological studies. But strains belong-
10 ing to the subspecies *lactis* are also important in cheese production. We therefore cloned and sequenced the *atpAGD_{L11}* genes from *L. lactis* subsp. *lactis*, (SEQ ID No. 6). Subsequently, a 4.2 kb fragment harbouring the *atpAGD_{L11}* genes was cloned into 5 vectors, pCP32, pCP34, pCP37, pCP41 and pCP44, resulting in the plasmids,
15 pCP32::*atpAGD_{L11}*, pCP34::*atpAGD_{L11}*, pCP37::*atpAGD_{L11}*, pCP41::*atpAGD_{L11}*, pCP44::*atpAGD_{L11}*, respectively. These plasmids were then transformed into *L. lactis* subsp. *lactis* as described in example 3. The resulting strains with
20 different expression levels of the F₁-ATPase subunits α , γ and β were then used to characterize the effect hereof on the growth yield, growth rate, glycolytic flux, and the cellular energy state of *L. lactis* subsp. *lactis*, as described in the examples 1-5.

25

EXAMPLE 7

Expression of F₁-ATPase subunits from *S. thermophilus*, ST3, in *S. thermophilus*, ST3

30

In the examples 3-6 above, we used strains of the genus *Lactococcus*. These strains are important in cheese production. As starter cultures for yogurt production, the dairy industry often uses strains of *S. thermophilus*. We
35 therefore cloned and sequenced the *atpAGD_{St}* genes from *S. thermophilus*, strain ST3 (SEQ ID No. 10). Subsequently, a 4.2 kb fragment harbouring the *atpAGD_{St}* genes was cloned

into the 5 vectors, pCP32, pCP34, pCP37, pCP41 and pCP44, resulting in the plasmids, pCP32::atpAGD_{St}, pCP34::atpAGD_{St}, pCP37::atpAGD_{St}, pCP41::atpAGD_{St}, pCP44::atpAGD_{St}, respectively. These plasmids were then transformed into *S. thermophilus* strain ST3. The resulting strains have different expression levels of the F₁-ATPase subunits α , γ , and β , and were then used to characterize the effect hereof on the growth yield, growth rate, glycolytic flux, and the cellular energy state of *S. thermophilus*, as described in the previous examples.

EXAMPLE 8

Expression of a truncated F₁-ATPase β subunit from *Phaffia rhodozyma* in *Saccharomyces cerevisiae*

In this example we show that uncoupled F₁-ATPase expression can also be used to hydrolyze ATP in yeast cells of *Saccharomyces cerevisiae*.

20

A cDNA gene library was prepared from total RNA, isolated from *Phaffia rhodozyma*, by cloning the cDNA fragments into the expression vector, pYES2.0. One of the resulting plasmids, pATPbeta, gave rise to an ade⁺ phenotype in the *Saccharomyces cerevisiae* strain, W301, which carries a mutation in the ADE2 gene. Sequencing of the clone revealed a 0.9 kb insert, which encoded a protein of 254 amino acids (SEQ ID No. 14). The encoded protein had a very high homology to the C-terminal part of F₁-ATPase β subunits from other organisms, prokaryotic as well as eukaryotic, including the β subunit from *S. cerevisiae* (86% identity).

The ADE2 mutation results in starvation for an intermediate further down in the purine metabolism, AICAR (which under normal conditions is produced by ADE3, two steps further down in this pathway). AICAR is essential for de

novo biosynthesis of AMP and GMP, and ADE2 mutants therefore need an alternative purine source in the growth medium. However, there is an alternative route for synthesis of AICAR which involves some of the genes involved in histidine biosynthesis. These genes are normally repressed under the conditions used for the complementation, but when the *HIS3* gene is introduced on a plasmid, this complements the ADE2 mutation because the cells start to produce AICAR. Since AICAR is a precursor for ATP, it is likely that a lack of ATP (or increased levels of ADP and AMP) provide a signal to derepress the *HIS3* gene and generate AICAR (which will subsequently end up as ATP). Indeed, cross-pathway regulation between purine and histidine biosynthesis has been found in yeast and involves the transcription factors BAS1 and BAS2. A reasonable explanation for the *ade'* phenotype conferred by the plasmid, is therefore that the plasmid gives rise to ATP hydrolysis in the cytoplasm, thereby effecting the concentrations of adenine nucleotides in the cytoplasm.

Importantly, this truncated β subunit from *Phaffia rhodozyma* that was encoded on pATPbeta, included the region of the β subunit which is thought to encode the catalytic site for ATP hydrolysis. The truncation of the N-terminal part of the β subunit probably means that the protein will no longer be exported into the mitochondrion, but should stay within the yeast cytoplasm.

The truncated β subunit pATPbeta is expressed from a *gal* promoter, i.e. it can be induced with galactose. If the truncated β subunit encoded by the clone is active in ATP hydrolysis it should result in a decrease in the growth yield, and at sufficiently high expression level, we should also observe inhibition of growth. The strain which expressed the truncated β subunit and a control strain (which contained a plasmid pHIS3 containing a *HIS3* gene from *Phaffia rhodozyma*), were streaked on plates

containing galactose as the energy source, which will give maximal expression of the truncated β subunit. Indeed, the growth of the strain which expressed the truncated β subunit was strongly inhibited by the presence of galactose, whereas the control strain grew normally. As a control, the growth of the two strains were also compared on a plate containing glucose as the energy source, conditions under which the expression of the β subunit should be repressed, and indeed we observed little difference in growth of the two strains on these plates, see table 6.

Subsequently, for the purpose of the physiological investigations, the two strains were converted into Rho^- strains (petit mutants, defective in oxidative phosphorylation) by standard treatment with ethidium bromide. The induction with galactose caused even stronger inhibition of growth in the Rho^- background, which further indicates that the cause of the growth inhibition is uncoupled ATP hydrolysis in the cytoplasm.

Table 6. Effect of expression of a truncated F_1 -ATPase β subunit from *Phaffia rhodozyma* in *S. cerevisiae* on SC plates

Strain/plasmid	SC-ura + glucose	SC-ura + galactose
Rho^+ /pATPbeta	+++++	+
Rho^+ /pHIS3	++++	+++
Rho^- /pATPbeta	+++++	-
Rho^- /pHIS3	++++	+++

Growth experiments were performed to measure the resulting changes in the ATP/ADP ratio and the degree of stimulation of the glycolytic flux and ethanol formation, essentially as described in the examples above, and to show that the truncated β subunit from *Phaffia rhodozyma* is active with respect to converting ATP into ADP in the yeast cell.

EXAMPLE 9.

Expression of F_1 -ATPase β subunit from *Trichoderma reesei* in *Saccharomyces cerevisiae*.

5

In this example we show that the expression of the F_1 -ATPase β subunit from the filamentous fungus, *Trichoderma reesei* can be used to improve the product formation of *Saccharomyces cerevisiae*.

10

The gene encoding the F_1 -ATPase β subunit homologue from *Trichoderma reesei* was isolated from a cDNA library, inserted into a multicopy expression vector, pAJ401. DNA sequencing (SEQ ID 16) revealed that the cloned gene had
15 very high homology to the β subunits from *Neurospora crassa* (91% identity), *Kluyveromyces lactis* (68%) and *Saccharomyces cerevisiae* (68%). Importantly, the first 43 amino acids in this β subunit, which encodes the signal for exporting the protein into the mitochondria, was ho-
20 mologous to the N-terminal part of the β subunit from *Neurospora crassa* (58% identity), but not to that of *Saccharomyces cerevisiae*. It is therefore likely that the β subunit from *Trichoderma reesei* will stay within the cytoplasm when expressed in *Saccharomyces cerevisiae*. This
25 is important for the many cases where the fermentation is carried out anaerobically, because in these cases it is probably most efficient if the ATP hydrolysis takes place in the cytoplasm. Alternatively, in those cases where the β subunit is transported into the mitochondrion, it may
30 be useful to genetically modify the β subunit so that it stays within the cytoplasm.

The gene encoding the F_1 -ATPase β subunit homologue from *Trichoderma reesei* was expressed in *S. cerevisiae* strain
35 VW1b (MAT alpha, leu2-3/112, ura3-52, trp1-289, his3D1, MAL2-8c, SUC2). To test whether the presence of the *T. reesei* β subunit resulted in ATP hydrolysis in the cyto-

plasm of the *Saccharomyces cerevisiae* host cells, we measured the intracellular concentrations of ATP, ADP and AMP, under various growth conditions in cultures of two strains expressing the β subunit (pATP β 34 and pATP β 44) and a strain carrying the vector plasmid, pFL60, see table 7.

Table 7. Effect of expression of *T. reesei* β subunit on ATP, ADP and AMP concentrations in *S. cerevisiae*

Strain	ATP $\mu\text{mol/gdw}$	ADP $\mu\text{mol/gdw}$	AMP $\mu\text{mol/gdw}$	ATP/ADP ratio
<i>Aerobic/exp.phase</i>				
pATP β 34	19.3	5.58	3.31	3.5
pATP β 44	13.9	5.15	3.25	2.7
pVECTOR	16.6	5.47	3.43	3.0
<i>Aerobic/stat.phase</i>				
pATP β 34	9.30	4.03	2.89	2.3
pATP β 44	8.99	3.90	2.42	2.3
pVECTOR	19.5	4.62	2.87	4.2
<i>anaerobic/stat.phase</i>				
pATP β 34	4.39	11.6	6.72	0.4
pATP β 44	3.14	10.5	6.65	0.3
pVECTOR	8.84	10.2	6.37	0.9

* according to Bergmeyer (1985)

The β subunit did not appear to have a significant effect on the concentrations of ATP, ADP and AMP in cells growing on glucose in the exponential growth phase. The reason is probably that the ATP concentration that the homeostatic control of ATP synthesis can here keep up with the extra drain on ATP conferred by the β subunit F_1 -ATPase activity. Indeed, the growth rate of these cultures was unaffected by the presence of the F_1 -ATPase activity, see table 7. But in the stationary cultures the concentration of ATP decreased significantly in the cultures expressing the β subunit, compared to the control. The effect was strongest in the anaerobically grown cultures where the ATP was lowered by a factor of 2-3. In these cultures, ATP must be generated through oxidative phosphorylation, (which is not even an option for the an-

aerobic cultures), and any effect of uncoupled ATP hydrolysis should therefore indeed be stronger in these cells.

- 5 Shake flask cultivations of cultures expressing the F_1 -ATPase β subunit homologue in *Saccharomyces cerevisiae*.

Shake flask cultivations were performed under microaerobic/anaerobic conditions with volume ratio 1/1.25 and no agitation; with 400 ml growth media in 500 ml Erlenmeyers on magnetic stirring. The growth media contained 5 g/l of glucose and amino acids and bases according to synthetic complete medium (SC-ura+0.5%G). OD_{600} was monitored during the cultivation ($OD_{600}=1.0$ is equal to 0.3 g/l dry weight). Ethanol and glucose were measured with HPLC (Waters, Sugar-Pak or IC-Pak columns). Production of ethanol (grams of ethanol per grams of cell dry weight) is shown in Table 8.

- 20 **Table 8.** Effect of expression of *T. reesei* β subunit, on fluxes of ethanol and glucose in *s. cerevisiae*

Strain	μ h^{-1}	J_{gluc} g/h/gdw	J_{etoh} g/h/gdw	J_{gluc} relative to control	J_{etoh} relative to control
pATP β 34	0.40	2.811	1.190	107.7	105.6
pATP β 44	0.40	2.750	1.187	105.3	105.3
pVECTOR control	0.39	2.611	1.127	100	100

- 25 These data show that the presence of the *T. reesei* F_1 -ATPase β subunit resulted in an increased flux of glucose, as well as ethanol, in the *Saccharomyces cerevisiae* host cells.

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SEQUENCE LISTING

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(2) INFORMATION FOR SEQ ID NO: 1:

- (1) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 4815 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: double
 - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: DNA (genomic)
- (iii) HYPOTHETICAL: NO
- (iv) ANTI-SENSE: NO
- (vi) ORIGINAL SOURCE:
 - (A) ORGANISM: Lactococcus lactis subsp. cremoris
 - (B) STRAIN: MG1363
- (ix) FEATURE:
 - (A) NAME/KEY: CDS
 - (B) LOCATION: 26..550
 - (D) OTHER INFORMATION: /codon_start= 26
/product= "ATPase subunit"
/gene= "atpH"
/standard_name= "delta subunit of the F1 portion of the FOF1 ATPase"
/label= delta-subunit
- (ix) FEATURE:
 - (A) NAME/KEY: CDS
 - (B) LOCATION: 742..2241
 - (D) OTHER INFORMATION: /codon_start= 742
/product= "ATPase subunit"
/gene= "atpA"
/standard_name= "alpha subunit of the F1 portion"

of the F₀F₁ ATPase"
/label= alpha-subunit

(ix) FEATURE:

(A) NAME/KEY: CDS
(B) LOCATION: 2260..3126
(D) OTHER INFORMATION: /codon_start= 2260
/product= "ATPase subunit"
/gene= "atpG"
/standard_name= "gamma subunit of the F₁ portion
of the F₀F₁ ATPase"
/label= gamma-subunit

(ix) FEATURE:

(A) NAME/KEY: CDS
(B) LOCATION: 3301..4707
(D) OTHER INFORMATION: /codon_start= 3301
/product= "ATPase subunit"
/gene= "atpD"
/standard_name= "beta subunit of the F₁ portion of
the F₀F₁ ATPase"
/label= beta-subunit

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

TATCTCGCTA AGTTAGGAGA ATAAG ATG ACA AAA GTA AAT TCA CAA AAA TAC	52
Met Thr Lys Val Asn Ser Gln Lys Tyr	
1 5	
AGT AAA GCT TTA CTT GAG GTC GCC CGA GAA AAA GGA CAA CTT GAA GCA	100
Ser Lys Ala Leu Leu Glu Val Ala Arg Glu Lys Gly Gln Leu Glu Ala	
10 15 20 25	
ATT CTT ACT GAA GTT AGC GAA ATG ATT CAG CTT TTC AAA GAA AAT AAC	148
Ile Leu Thr Glu Val Ser Glu Met Ile Gln Leu Phe Lys Glu Asn Asn	
30 35 40	
TTA GGT GCT TTT TTA GCA AAT GAA GTT TAT TCA TTC TCT GCT AAA TCT	196
Leu Gly Ala Phe Leu Ala Asn Glu Val Tyr Ser Phe Ser Ala Lys Ser	
45 50 55	
GAA TTG ATT GAT ACT TTG CTT CAA ACT TCA TCA GAA GTG ATG TCA AAT	244
Glu Leu Ile Asp Thr Leu Leu Gln Thr Ser Ser Glu Val Met Ser Asn	
60 65 70	
TTC CTG AAT ACT ATT CGT TCT AAT GGA CGT CTA GCT GAC CTC GGA GAA	292
Phe Leu Asn Thr Ile Arg Ser Asn Gly Arg Leu Ala Asp Leu Gly Glu	
75 80 85	
ATA CTT GAA GAA ACT AAA AAT GCA GCA GAT GAC ATG TTC AAA ATT GCT	340
Ile Leu Glu Glu Thr Lys Asn Ala Ala Asp Asp Met Phe Lys Ile Ala	
90 95 100 105	
GAC GTT GAA GTT GTT TCA AGT ATT GCA TTG TCA GAA GCT CAA ATT GAA	388
Asp Val Glu Val Val Ser Ser Ile Ala Leu Ser Glu Ala Gln Ile Glu	
110 115 120	
AAA TTT AAA GCA ATG GCT AAA TCA AAA TTT GAT TTA AAC GAA GTA ACA	436
Lys Phe Lys Ala Met Ala Lys Ser Lys Phe Asp Leu Asn Glu Val Thr	
125 130 135	

GTA ATT AAT ACA GTC AAT GAA AAA ATT CTC GGA GGA TTC ATT GTG AAC Val Ile Asn Thr Val Asn Glu Lys Ile Leu Gly Gly Phe Ile Val Asn 140 145 150	484
TCT CGT GGA AAA ATT ATT GAC GCC TCA TTA AAA ACA CAA TTG GCT AAA Ser Arg Gly Lys Ile Ile Asp Ala Ser Leu Lys Thr Gln Leu Ala Lys 155 160 165	532
ATC GCC GCT GAA ATC CTC TAATCAGGAT AGAAAAATTT TCTTCCTTTG Ile Ala Ala Glu Ile Leu 170 175	580
TTAAAACTT AGTGGAGAAT TTTTCAAAC TAACTGTTA AACTTTTGAA AACATGCAAA	640
GGTAATTTTA AAACCTTGCTT ATTCATGCTC AAAAAGTATA ACTGCAGTTT AAAGCTAAAT	700
AGCCTTGAAC TAGTAAAAAA TTTCTAGAAG GGAGCATATT T TTG GCA ATT AAA Leu Ala Ile Lys 1	753
GCT AAT GAA ATC AGC TCA CTG ATT AAA AAA CAA ATT GAA AAT TTC ACA Ala Asn Glu Ile Ser Ser Leu Ile Lys Lys Gln Ile Glu Asn Phe Thr 5 10 15 20	801
CCA GAT TTT GAA GTT GCT GAA ACT GGT GTC GTT ACC TAT GTT GGT GAT Pro Asp Phe Glu Val Ala Glu Thr Gly Val Val Thr Tyr Val Gly Asp 25 30 35	849
GGT ATC GCG CGT GCC TAT GGC CTT GAA AAT GCG ATG AGC GGT GAG CTT Gly Ile Ala Arg Ala Tyr Gly Leu Glu Asn Ala Met Ser Gly Glu Leu 40 45 50	897
GTT GAG TTT TCA AAT GGT ATA CTT GGT ATG GCG CAA AAC TTG GAT GCT Val Glu Phe Ser Asn Gly Ile Leu Gly Met Ala Gln Asn Leu Asp Ala 55 60 65	945
ACA GAC GTT GGT ATT ATC GTA CTT GGT GAT TTC CTC TCA ATT CGT GAA Thr Asp Val Gly Ile Ile Val Leu Gly Asp Phe Leu Ser Ile Arg Glu 70 75 80	993
GGT GAC ACT GTT AAA CGT ACA GGT AAA ATC ATG GAA ATC CAA GTT GGT Gly Asp Thr Val Lys Arg Thr Gly Lys Ile Met Glu Ile Gln Val Gly 85 90 95 100	1041
GAA GAA CTC ATC GGA CGT GTT GTA AAC CCA CTT GGA CAA CCC GTT GAT Glu Glu Leu Ile Gly Arg Val Val Asn Pro Leu Gly Gln Pro Val Asp 105 110 115	1089
GGA CTT GGA GAA CTT AAT ACA GGT AAA ACT CGT CCA GTT GAA GCA AAA Gly Leu Gly Glu Leu Asn Thr Gly Lys Thr Arg Pro Val Glu Ala Lys 120 125 130	1137
GCT CCT GGT GTT ATG CAA CGT AAA TCA GTC TCT GAG CCA TTA CAA ACT Ala Pro Gly Val Met Gln Arg Lys Ser Val Ser Glu Pro Leu Gln Thr 135 140 145	1185
GGT CTT AAA GCG ATT GAT GCC CTC GTT CCA ATT GGA CGT GGA CAA CGT Gly Leu Lys Ala Ile Asp Ala Leu Val Pro Ile Gly Arg Gly Gln Arg 150 155 160	1233

GAA TTA ATT ATC GGA GAC CGT CAA ACT GGT AAA ACT TCA GTC GCT ATT Glu Leu Ile Ile Gly Asp Arg Gln Thr Gly Lys Thr Ser Val Ala Ile 165 170 175 180	1281
GAT GCA ATC TTG AAC CAA AAA GGT CAA GAT ATG ATT TGT ATC TAT GTT Asp Ala Ile Leu Asn Gln Lys Gly Gln Asp Met Ile Cys Ile Tyr Val 185 190 195	1329
GCG ATT GGA CAA AAA GAG TCA ACA GTT CGT ACA CAA GTT GAA ACG CTC Ala Ile Gly Gln Lys Glu Ser Thr Val Arg Thr Gln Val Glu Thr Leu 200 205 210	1377
CGT AAA CTC GGT GCG ATG GAT TAT ACA ATC GTC GTA ACT GCG TCA GCT Arg Lys Leu Gly Ala Met Asp Tyr Thr Ile Val Val Thr Ala Ser Ala 215 220 225	1425
TCT CAA CCT TCT CCA CTC CTT TAC ATC GCT CCT TAC GCT GGA GCT GCA Ser Gln Pro Ser Pro Leu Leu Tyr Ile Ala Pro Tyr Ala Gly Ala Ala 230 235 240	1473
ATG GGT GAA GAA TTT ATG TAT AAC GGT AAA CAT GTC TTG GTT GTT TAT Met Gly Glu Glu Phe Met Tyr Asn Gly Lys His Val Leu Val Val Tyr 245 250 255 260	1521
GAT GAT TTA TCT AAA CAA GCG GTC GCT TAC CGT GAA CTT TCT CTC TTG Asp Asp Leu Ser Lys Gln Ala Val Ala Tyr Arg Glu Leu Ser Leu Leu 265 270 275	1569
CTC CGT CGT CCA CCA GGT CGT GAA GCA TAC CCA GGT GAC GTT TTC TAC Leu Arg Arg Pro Pro Gly Arg Glu Ala Tyr Pro Gly Asp Val Phe Tyr 280 285 290	1617
TTG CAC TCA CGT CTT TTG GAA CGT GCT GCT AAA TTG TCT GAT GAT CTT Leu His Ser Arg Leu Leu Glu Arg Ala Ala Lys Leu Ser Asp Asp Leu 295 300 305	1665
GGT GGT GGA TCA ATG ACG GCT TTG CCA TTC ATT GAA ACA CAA GCA GGT Gly Gly Gly Ser Met Thr Ala Leu Pro Phe Ile Glu Thr Gln Ala Gly 310 315 320	1713
GAT ATC TCA GCT TAT ATT CCA ACA AAC GTT ATC TCT ATT ACC GAC GGT Asp Ile Ser Ala Tyr Ile Pro Thr Asn Val Ile Ser Ile Thr Asp Gly 325 330 335 340	1761
CAA ATT TTC CTT GAA AAT GAC TTG TTC TAT TCA GGT GTA CGT CCT GCC Gln Ile Phe Leu Glu Asn Asp Leu Phe Tyr Ser Gly Val Arg Pro Ala 345 350 355	1809
ATT GAT GCT GGT TCA TCA GTA TCA CGT GTT GGT GGT GCC GCA CAA ATC Ile Asp Ala Gly Ser Ser Val Ser Arg Val Gly Gly Ala Ala Gln Ile 360 365 370	1857
AAA GCC ATG AAG AAA GTA GCT GGT ACT TTG CGT CTT GAC CTT GCG TCG Lys Ala Met Lys Lys Val Ala Gly Thr Leu Arg Leu Asp Leu Ala Ser 375 380 385	1905
TTC CGT GAA CTT GAA GCC TTT ACA CAA TTT GGT TCT GAC CTT GAT GAA Phe Arg Glu Leu Glu Ala Phe Thr Gln Phe Gly Ser Asp Leu Asp Glu 390 395 400	1953
GCG ACT CAA GCA AAA TTG AAT CGT GGT CGT CGT ACC GTT GAA GTC TTG Ala Thr Gln Ala Lys Leu Asn Arg Gly Arg Arg Thr Val Glu Val Leu 405 410 415 420	2001

AAA CAA CCA TTG CAC AAA CCA TTG GCT GTT GAA AAA CAA GTT TTG ATT Lys Gln Pro Leu His Lys Pro Leu Ala Val Glu Lys Gln Val Leu Ile 425 430 435	2049
CTC TAT GCA TTG ACT CAT GGT CAT CTT GAT AAT GTT CCA GTT GAT GAT Leu Tyr Ala Leu Thr His Gly His Leu Asp Asn Val Pro Val Asp Asp 440 445 450	2097
GTT CTT GAT TTT GAA ACT AAA ATG TTC GAT TTC TTC GAT GCA AAT TAT Val Leu Asp Phe Glu Thr Lys Met Phe Asp Phe Asp Ala Asn Tyr 455 460 465	2145
GCA GAT CTC TTG AAC GTA ATT ACT GAC ACT AAA GAT TTG CCA GAA GAA Ala Asp Leu Leu Asn Val Ile Thr Asp Thr Lys Asp Leu Pro Glu Glu 470 475 480	2193
GCA AAA CTT GAC GAA GCA ATT AAA GCA TTC AAA AAT ACA ACG AAT TAT Ala Lys Leu Asp Glu Ala Ile Lys Ala Phe Lys Asn Thr Thr Asn Tyr 485 490 495 500	2241
TAATAAGGAG GCTAACTA ATG GGA GCT TCA CTT AAC GAA ATA AAA ACT AAG Met Gly Ala Ser Leu Asn Glu Ile Lys Thr Lys 1 5 10	2292
ATT GCG TCA ACA AAG AAA ACA AGT CAA ATC ACA GGT GCC ATG CAA ATG Ile Ala Ser Thr Lys Lys Thr Ser Gln Ile Thr Gly Ala Met Gln Met 15 20 25	2340
GTT TCT GCT GCT AAA CTT CAA AAA GCA GAA TCT CAC GCT AAA GCT TTT Val Ser Ala Ala Lys Leu Gln Lys Ala Glu Ser His Ala Lys Ala Phe 30 35 40	2388
CAG ACT TAT GCT GAA AAA GTA CGT AAG ATT ACG ACT GAC TTA GTT TCA Gln Thr Tyr Ala Glu Lys Val Arg Lys Ile Thr Thr Asp Leu Val Ser 45 50 55	2436
AGC GAT AAT GAG CCG GCC AAA AAT CCG ATG ATG ATT AAA CGT GAA GTC Ser Asp Asn Glu Pro Ala Lys Asn Pro Met Met Ile Lys Arg Glu Val 60 65 70 75	2484
AAG AAA ACT GGC TAT CTC GTT ATC ACA TCA GAT CGT GGG CTT GTT GGC Lys Lys Thr Gly Tyr Leu Val Ile Thr Ser Asp Arg Gly Leu Val Gly 80 85 90	2532
AGT TAT AAT TCA AAT ATT TTG AAG TCT GTT ATA AGT AAT ATA CGT AAA Ser Tyr Asn Ser Asn Ile Leu Lys Ser Val Ile Ser Asn Ile Arg Lys 95 100 105	2580
CGC CAC ACA AAT GAG AGT GAG TAT ACA ATA CTT GCC CTT GGT GGT ACG Arg His Thr Asn Glu Ser Glu Tyr Thr Ile Leu Ala Leu Gly Gly Thr 110 115 120	2628
GGA GCG GAC TTT TTC AAA GCC CGT AAC GTC AAA GTT TCT TAT GTT CTT Gly Ala Asp Phe Phe Lys Ala Arg Asn Val Lys Val Ser Tyr Val Leu 125 130 135	2676
CGC GGA CTT TCA GAT CAA CCG ACC TTT GAA GAG GTT CGG GCA ATT GTT Arg Gly Leu Ser Asp Gln Pro Thr Phe Glu Glu Val Arg Ala Ile Val 140 145 150 155	2724
ACA GAA GCC GTA GAA GAA TAT CAA GCA GAA GAA TTC GAT GAA CTC TAT Thr Glu Ala Val Glu Glu Tyr Gln Ala Glu Glu Phe Asp Glu Leu Tyr 160 165 170	2772

GTT TGT TAC AAC CAC CAT GTG AAC TCA TTG GTA AGT GAG GCA CGG ATG Val Cys Tyr Asn His His Val Asn Ser Leu Val Ser Glu Ala Arg Met 175 180 185	2820
GAA AAA ATG TTA CCT ATT TCT TTT GAT GAA AAA GGT GAC GAA AAA GCA Glu Lys Met Leu Pro Ile Ser Phe Asp Glu Lys Gly Asp Glu Lys Ala 190 195 200	2868
TCT CTT GTT ACA TTT GAA TTA GAA CCA GAT CGT GAA ACA ATC TTA AAT Ser Leu Val Thr Phe Glu Leu Glu Pro Asp Arg Glu Thr Ile Leu Asn 205 210 215	2916
CAG TTG TTG CCG CAA TAT GCT GAA AGT ATG ATT TAT GGC TCA ATT GTT Gln Leu Leu Pro Gln Tyr Ala Glu Ser Met Ile Tyr Gly Ser Ile Val 220 225 230 235	2964
GAT GCA AAA ACA GCA GAA CAT GCT GCA GGT ATG ACC GCA ATG CGT ACT Asp Ala Lys Thr Ala Glu His Ala Ala Gly Met Thr Ala Met Arg Thr 240 245 250	3012
GCA ACA GAT AAT GCA CAT TCT GTC ATT AAT GAT TTA ACC ATT CAA TAT Ala Thr Asp Asn Ala His Ser Val Ile Asn Asp Leu Thr Ile Gln Tyr 255 260 265	3060
AAC CGT GCT CGT CAA GCT TCA ATT ACG CAA GAA ATT ACG GAA ATT GTT Asn Arg Ala Arg Gln Ala Ser Ile Thr Gln Glu Ile Thr Glu Ile Val 270 275 280	3108
GCG GGT GCT TCA GCG CTA TAATTACTGT CAAACATTAT TCTCAATGTT Ala Gly Ala Ser Ala Leu 285	3156
ACGATTTATC AACTTGAGGA ATAAATGTTT TGTCAGTAAA GGCTTTGAAT TTAAATACG	3216
TTTGTCAGTA AATTTTACT GATTAGCTTA AAAATGAATA GAAATTCTGT TGTTAGACAG	3276
AAAATAAAAA CAGGAGGAAA AACA TTG AGT TCT GGT AAA ATT ACT CAG GTT Leu Ser Ser Gly Lys Ile Thr Gln Val 1 5	3327
ATC GGT CCC GTC GTT GAC GTG GAA TTT GGT TCT GAT GCC AAA CTG CCT Ile Gly Pro Val Val Asp Val Glu Phe Gly Ser Asp Ala Lys Leu Pro 10 15 20 25	3375
GAG ATT AAC AAT GCC TTG ATT GTC TAC AAA GAT GTC AAT GGT TTA AAA Glu Ile Asn Asn Ala Leu Ile Val Tyr Lys Asp Val Asn Gly Leu Lys 30 35 40	3423
ACA AAA ATT ACT CTT GAA GTT GCT TTG GAA CTT GGT GAT GGT GCA GTT Thr Lys Ile Thr Leu Glu Val Ala Leu Glu Leu Gly Asp Gly Ala Val 45 50 55	3471
CGT ACG ATC GCT ATG GAA TCT ACT GAT GGA TTG ACT CGT GGA CTT GAA Arg Thr Ile Ala Met Glu Ser Thr Asp Gly Leu Thr Arg Gly Leu Glu 60 65 70	3519
GTC CTT GAT ACA GGT AAA GCG GTC AGC GTT CCT GTT GGT GAA TCT ACT Val Leu Asp Thr Gly Lys Ala Val Ser Val Pro Val Gly Glu Ser Thr 75 80 85	3567
CTT GGT CGT GTT TTT AAT GTC CTT GGT GAC GTT ATT GAT GGT GGA GAA Leu Gly Arg Val Phe Asn Val Leu Gly Asp Val Ile Asp Gly Gly Glu 90 95 100 105	3615

GAT TTC CCT GCT GAT GCA GAA CGT AAT CCT ATC CAC AAG AAA GCT CCA Asp Phe Pro Ala Asp Ala Glu Arg Asn Pro Ile His Lys Lys Ala Pro 110 115 120	3663
ACT TTT GAC GAA TTG TCA ACT GCA AAT GAA GTT CTT GTA ACA GGG ATT Thr Phe Asp Glu Leu Ser Thr Ala Asn Glu Val Leu Val Thr Gly Ile 125 130 135	3711
AAA GTT GTC GAT TTA CTT GCC CCT TAT CTT AAA GGT GGG AAA GTC GGA Lys Val Val Asp Leu Leu Ala Pro Tyr Leu Lys Gly Gly Lys Val Gly 140 145 150	3759
CTC TTC GGT GGT GCC GGT GTT GGT AAA ACC GTC CTT ATC CAA GAA TTG Leu Phe Gly Gly Ala Gly Val Gly Lys Thr Val Leu Ile Gln Glu Leu 155 160 165	3807
ATT CAC AAT ATT GCC CAA GAA CAC GGT GGT ATT TCT GTA TTT ACA GGT Ile His Asn Ile Ala Gln Glu His Gly Gly Ile Ser Val Phe Thr Gly 170 175 180 185	3855
GTT GGC GAT CGT ACT CGT GAC GGG AAT GAC CTT TAC TGG GAA ATG AAA Val Gly Asp Arg Thr Arg Asp Gly Asn Asp Leu Tyr Trp Glu Met Lys 190 195 200	3903
GAA TCA GGC GTT ATT GAA AAA ACA GCC ATG GTC TTT GGT CAA ATG AAT Glu Ser Gly Val Ile Glu Lys Thr Ala Met Val Phe Gly Gln Met Asn 205 210 215	3951
GAA CCA CCT GGA GCA CGT ATG CGT GTT GCC CTT ACT GGT TTA ACA ATT Glu Pro Pro Gly Ala Arg Met Arg Val Ala Leu Thr Gly Leu Thr Ile 220 225 230	3999
GCG GAA TAT TTC CGT GAT GTT CAA GGA CAA GAC GTA TTG CTT TTC ATC Ala Glu Tyr Phe Arg Asp Val Gln Gly Gln Asp Val Leu Leu Phe Ile 235 240 245	4047
GAT AAC ATC TTC CGT TTC ACT CAA GCT GGT TCA GAA GTT TCT GCC CTT Asp Asn Ile Phe Arg Phe Thr Gln Ala Gly Ser Glu Val Ser Ala Leu 250 255 260 265	4095
TGG GGA CGT ATG CCT TCT GCC GTT GGT TAC CAA CCA ACT CTT GCA ACT Trp Gly Arg Met Pro Ser Ala Val Gly Tyr Gln Pro Thr Leu Ala Thr 270 275 280	4143
GAA ATG GTT CAA TTA CAG GAA CGT ATC ACT TCT ACT AAG AAG GGT TCT Glu Met Val Gln Leu Gln Glu Arg Ile Thr Ser Thr Lys Lys Gly Ser 285 290 295	4191
GTT ACA TCT ATC CCA GCG ATT TAT GTC CCT GCC GAT GAC TAT ACT GAC Val Thr Ser Ile Pro Ala Ile Tyr Val Pro Ala Asp Asp Tyr Thr Asp 300 305 310	4239
CCA GCG CCA GCT ACA GCC TTC GCT CAC TTG GAC GCA ACA ACT AAC TTG Pro Ala Pro Ala Thr Ala Phe Ala His Leu Asp Ala Thr Thr Asn Leu 315 320 325	4287
GAA CGT CGT TTG ACA CAA ATG GGT ATC TAT CCA GCC GTT GAC CCA CTT Glu Arg Arg Leu Thr Gln Met Gly Ile Tyr Pro Ala Val Asp Pro Leu 330 335 340 345	4335
GCT TCA TCA TCA CGT GCG CTT ACA CCT GAA ATT GTT GGT GAA GAA CAC Ala Ser Ser Ser Arg Ala Leu Thr Pro Glu Ile Val Gly Glu Glu His 350 355 360	4383

TAT GAA GTT GCA ATG GAA GTT CAA CGT GTC CTT CAA CGC TAC AAA GAA 4431
 Tyr Glu Val Ala Met Glu Val Gln Arg Val Leu Gln Arg Tyr Lys Glu
 365 370 375
 TTG CAA GAT ATC ATT GCC ATT CTT GGT ATG GAT GAA TTG TCA GAT GAT 4479
 Leu Gln Asp Ile Ile Ala Ile Leu Gly Met Asp Glu Leu Ser Asp Asp
 380 385 390
 GAA AAA ATT CTC GTT GGA CGT GCA CGT CGT ATC CAA TTC TTC CTT TCA 4527
 Glu Lys Ile Leu Val Gly Arg Ala Arg Arg Ile Gln Phe Phe Leu Ser
 395 400 405
 CAA AAC TTC CAC GTT GCT GAA CAG TTT ACT GGT CAA CCT GGT TCA TAT 4575
 Gln Asn Phe His Val Ala Glu Gln Phe Thr Gly Gln Pro Gly Ser Tyr
 410 415 420 425
 GTA CCA ATT GAC AAA ACA GTT CAT GAC TTC AAG GAA ATT TTG GAA GGT 4623
 Val Pro Ile Asp Lys Thr Val His Asp Phe Lys Glu Ile Leu Glu Gly
 430 435 440
 AAA TAT GAC GAA GTC CCT GAA GAT GCT TTC CGT GGA GTA GGT CCA ATT 4671
 Lys Tyr Asp Glu Val Pro Glu Asp Ala Phe Arg Gly Val Gly Pro Ile
 445 450 455
 GAA GAC GTA CTT GCA AAA GCA AAA TCA ATG GGT TAT TAATTGATT 4717
 Glu Asp Val Leu Ala Lys Ala Lys Ser Met Gly Tyr
 460 465
 TCTTATGAAA TGACAAAGTG AAAATACATT ATTGAATCGC AAAATTTACT GACAATAATT 4777
 CTGTCGTAAG TGCTCACTTT TAAGTTGTTC CGATCGTT 4815

(2) INFORMATION FOR SEQ ID NO: 2:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 175 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

Met Thr Lys Val Asn Ser Gln Lys Tyr Ser Lys Ala Leu Leu Glu Val
 1 5 10 15
 Ala Arg Glu Lys Gly Gln Leu Glu Ala Ile Leu Thr Glu Val Ser Glu
 20 25 30
 Met Ile Gln Leu Phe Lys Glu Asn Asn Leu Gly Ala Phe Leu Ala Asn
 35 40 45
 Glu Val Tyr Ser Phe Ser Ala Lys Ser Glu Leu Ile Asp Thr Leu Leu
 50 55 60
 Gln Thr Ser Ser Glu Val Met Ser Asn Phe Leu Asn Thr Ile Arg Ser
 65 70 75 80
 Asn Gly Arg Leu Ala Asp Leu Gly Glu Ile Leu Glu Glu Thr Lys Asn
 85 90 95
 Ala Ala Asp Asp Met Phe Lys Ile Ala Asp Val Glu Val Val Ser Ser
 100 105 110

Ile Ala Leu Ser Glu Ala Gln Ile Glu Lys Phe Lys Ala Met Ala Lys
 115 120 125
 Ser Lys Phe Asp Leu Asn Glu Val Thr Val Ile Asn Thr Val Asn Glu
 130 135 140
 Lys Ile Leu Gly Gly Phe Ile Val Asn Ser Arg Gly Lys Ile Ile Asp
 145 150 155 160
 Ala Ser Leu Lys Thr Gln Leu Ala Lys Ile Ala Ala Glu Ile Leu
 165 170 175

(2) INFORMATION FOR SEQ ID NO: 3:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 500 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

Leu Ala Ile Lys Ala Asn Glu Ile Ser Ser Leu Ile Lys Lys Gln Ile
 1 5 10 15
 Glu Asn Phe Thr Pro Asp Phe Glu Val Ala Glu Thr Gly Val Val Thr
 20 25 30
 Tyr Val Gly Asp Gly Ile Ala Arg Ala Tyr Gly Leu Glu Asn Ala Met
 35 40 45
 Ser Gly Glu Leu Val Glu Phe Ser Asn Gly Ile Leu Gly Met Ala Gln
 50 55 60
 Asn Leu Asp Ala Thr Asp Val Gly Ile Ile Val Leu Gly Asp Phe Leu
 65 70 75 80
 Ser Ile Arg Glu Gly Asp Thr Val Lys Arg Thr Gly Lys Ile Met Glu
 85 90 95
 Ile Gln Val Gly Glu Glu Leu Ile Gly Arg Val Val Asn Pro Leu Gly
 100 105 110
 Gln Pro Val Asp Gly Leu Gly Glu Leu Asn Thr Gly Lys Thr Arg Pro
 115 120 125
 Val Glu Ala Lys Ala Pro Gly Val Met Gln Arg Lys Ser Val Ser Glu
 130 135 140
 Pro Leu Gln Thr Gly Leu Lys Ala Ile Asp Ala Leu Val Pro Ile Gly
 145 150 155 160
 Arg Gly Gln Arg Glu Leu Ile Ile Gly Asp Arg Gln Thr Gly Lys Thr
 165 170 175
 Ser Val Ala Ile Asp Ala Ile Leu Asn Gln Lys Gly Gln Asp Met Ile
 180 185 190
 Cys Ile Tyr Val Ala Ile Gly Gln Lys Glu Ser Thr Val Arg Thr Gln
 195 200 205

Val Glu Thr Leu Arg Lys Leu Gly Ala Met Asp Tyr Thr Ile Val Val
 210 215 220
 Thr Ala Ser Ala Ser Gln Pro Ser Pro Leu Leu Tyr Ile Ala Pro Tyr
 225 230 235 240
 Ala Gly Ala Ala Met Gly Glu Glu Phe Met Tyr Asn Gly Lys His Val
 245 250 255
 Leu Val Val Tyr Asp Asp Leu Ser Lys Gln Ala Val Ala Tyr Arg Glu
 260 265 270
 Leu Ser Leu Leu Leu Arg Arg Pro Pro Gly Arg Glu Ala Tyr Pro Gly
 275 280 285
 Asp Val Phe Tyr Leu His Ser Arg Leu Leu Glu Arg Ala Ala Lys Leu
 290 295 300
 Ser Asp Asp Leu Gly Gly Gly Ser Met Thr Ala Leu Pro Phe Ile Glu
 305 310 315 320
 Thr Gln Ala Gly Asp Ile Ser Ala Tyr Ile Pro Thr Asn Val Ile Ser
 325 330 335
 Ile Thr Asp Gly Gln Ile Phe Leu Glu Asn Asp Leu Phe Tyr Ser Gly
 340 345 350
 Val Arg Pro Ala Ile Asp Ala Gly Ser Ser Val Ser Arg Val Gly Gly
 355 360 365
 Ala Ala Gln Ile Lys Ala Met Lys Lys Val Ala Gly Thr Leu Arg Leu
 370 375 380
 Asp Leu Ala Ser Phe Arg Glu Leu Glu Ala Phe Thr Gln Phe Gly Ser
 385 390 395 400
 Asp Leu Asp Glu Ala Thr Gln Ala Lys Leu Asn Arg Gly Arg Arg Thr
 405 410 415
 Val Glu Val Leu Lys Gln Pro Leu His Lys Pro Leu Ala Val Glu Lys
 420 425 430
 Gln Val Leu Ile Leu Tyr Ala Leu Thr His Gly His Leu Asp Asn Val
 435 440 445
 Pro Val Asp Asp Val Leu Asp Phe Glu Thr Lys Met Phe Asp Phe Phe
 450 455 460
 Asp Ala Asn Tyr Ala Asp Leu Leu Asn Val Ile Thr Asp Thr Lys Asp
 465 470 475 480
 Leu Pro Glu Glu Ala Lys Leu Asp Glu Ala Ile Lys Ala Phe Lys Asn
 485 490 495
 Thr Thr Asn Tyr
 500

(2) INFORMATION FOR SEQ ID NO: 4:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 289 amino acids
 (B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

```

Met Gly Ala Ser Leu Asn Glu Ile Lys Thr Lys Ile Ala Ser Thr Lys
 1           5           10           15
Lys Thr Ser Gln Ile Thr Gly Ala Met Gln Met Val Ser Ala Ala Lys
          20           25           30
Leu Gln Lys Ala Glu Ser His Ala Lys Ala Phe Gln Thr Tyr Ala Glu
          35           40           45
Lys Val Arg Lys Ile Thr Thr Asp Leu Val Ser Ser Asp Asn Glu Pro
          50           55           60
Ala Lys Asn Pro Met Met Ile Lys Arg Glu Val Lys Lys Thr Gly Tyr
          65           70           75           80
Leu Val Ile Thr Ser Asp Arg Gly Leu Val Gly Ser Tyr Asn Ser Asn
          85           90           95
Ile Leu Lys Ser Val Ile Ser Asn Ile Arg Lys Arg His Thr Asn Glu
          100          105          110
Ser Glu Tyr Thr Ile Leu Ala Leu Gly Gly Thr Gly Ala Asp Phe Phe
          115          120          125
Lys Ala Arg Asn Val Lys Val Ser Tyr Val Leu Arg Gly Leu Ser Asp
          130          135          140
Gln Pro Thr Phe Glu Glu Val Arg Ala Ile Val Thr Glu Ala Val Glu
          145          150          155          160
Glu Tyr Gln Ala Glu Glu Phe Asp Glu Leu Tyr Val Cys Tyr Asn His
          165          170          175
His Val Asn Ser Leu Val Ser Glu Ala Arg Met Glu Lys Met Leu Pro
          180          185          190
Ile Ser Phe Asp Glu Lys Gly Asp Glu Lys Ala Ser Leu Val Thr Phe
          195          200          205
Glu Leu Glu Pro Asp Arg Glu Thr Ile Leu Asn Gln Leu Leu Pro Gln
          210          215          220
Tyr Ala Glu Ser Met Ile Tyr Gly Ser Ile Val Asp Ala Lys Thr Ala
          225          230          235          240
Glu His Ala Ala Gly Met Thr Ala Met Arg Thr Ala Thr Asp Asn Ala
          245          250          255
His Ser Val Ile Asn Asp Leu Thr Ile Gln Tyr Asn Arg Ala Arg Gln
          260          265          270
Ala Ser Ile Thr Gln Glu Ile Thr Glu Ile Val Ala Gly Ala Ser Ala
          275          280          285
Leu

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(2) INFORMATION FOR SEQ ID NO: 5:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 469 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5:

```

Leu Ser Ser Gly Lys Ile Thr Gln Val Ile Gly Pro Val Val Asp Val
 1           5           10           15
Glu Phe Gly Ser Asp Ala Lys Leu Pro Glu Ile Asn Asn Ala Leu Ile
          20           25           30
Val Tyr Lys Asp Val Asn Gly Leu Lys Thr Lys Ile Thr Leu Glu Val
          35           40           45
Ala Leu Glu Leu Gly Asp Gly Ala Val Arg Thr Ile Ala Met Glu Ser
          50           55           60
Thr Asp Gly Leu Thr Arg Gly Leu Glu Val Leu Asp Thr Gly Lys Ala
          65           70           75           80
Val Ser Val Pro Val Gly Glu Ser Thr Leu Gly Arg Val Phe Asn Val
          85           90           95
Leu Gly Asp Val Ile Asp Gly Gly Glu Asp Phe Pro Ala Asp Ala Glu
          100          105          110
Arg Asn Pro Ile His Lys Lys Ala Pro Thr Phe Asp Glu Leu Ser Thr
          115          120          125
Ala Asn Glu Val Leu Val Thr Gly Ile Lys Val Val Asp Leu Leu Ala
          130          135          140
Pro Tyr Leu Lys Gly Gly Lys Val Gly Leu Phe Gly Gly Ala Gly Val
          145          150          155          160
Gly Lys Thr Val Leu Ile Gln Glu Leu Ile His Asn Ile Ala Gln Glu
          165          170          175
His Gly Gly Ile Ser Val Phe Thr Gly Val Gly Asp Arg Thr Arg Asp
          180          185          190
Gly Asn Asp Leu Tyr Trp Glu Met Lys Glu Ser Gly Val Ile Glu Lys
          195          200          205
Thr Ala Met Val Phe Gly Gln Met Asn Glu Pro Pro Gly Ala Arg Met
          210          215          220
Arg Val Ala Leu Thr Gly Leu Thr Ile Ala Glu Tyr Phe Arg Asp Val
          225          230          235          240
Gln Gly Gln Asp Val Leu Leu Phe Ile Asp Asn Ile Phe Arg Phe Thr
          245          250          255
Gln Ala Gly Ser Glu Val Ser Ala Leu Trp Gly Arg Met Pro Ser Ala
          260          265          270
Val Gly Tyr Gln Pro Thr Leu Ala Thr Glu Met Val Gln Leu Gln Glu
          275          280          285

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Arg Ile Thr Ser Thr Lys Lys Gly Ser Val Thr Ser Ile Pro Ala Ile
290                295                300

Tyr Val Pro Ala Asp Asp Tyr Thr Asp Pro Ala Pro Ala Thr Ala Phe
305                310                315                320

Ala His Leu Asp Ala Thr Thr Asn Leu Glu Arg Arg Leu Thr Gln Met
                325                330                335

Gly Ile Tyr Pro Ala Val Asp Pro Leu Ala Ser Ser Ser Arg Ala Leu
                340                345                350

Thr Pro Glu Ile Val Gly Glu Glu His Tyr Glu Val Ala Met Glu Val
                355                360                365

Gln Arg Val Leu Gln Arg Tyr Lys Glu Leu Gln Asp Ile Ile Ala Ile
                370                375                380

Leu Gly Met Asp Glu Leu Ser Asp Asp Glu Lys Ile Leu Val Gly Arg
385                390                395                400

Ala Arg Arg Ile Gln Phe Phe Leu Ser Gln Asn Phe His Val Ala Glu
                405                410                415

Gln Phe Thr Gly Gln Pro Gly Ser Tyr Val Pro Ile Asp Lys Thr Val
                420                425                430

His Asp Phe Lys Glu Ile Leu Glu Gly Lys Tyr Asp Glu Val Pro Glu
                435                440                445

Asp Ala Phe Arg Gly Val Gly Pro Ile Glu Asp Val Leu Ala Lys Ala
450                455                460

Lys Ser Met Gly Tyr
465

```

(2) INFORMATION FOR SEQ ID NO: 6:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 2207 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: *Lactococcus lactis* subsp. *lactis*

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 4..633
- (D) OTHER INFORMATION: /partial
 - /codon_start= 4
 - /product= "ATPase subunit, partial sequence"
 - /gene= "atpA"
 - /standard_name= "alpha subunit of the F1 portion of the F0F1 ATPase"
 - /label= alpha-subunit

(ix) FEATURE:

(A) NAME/KEY: CDS
 (B) LOCATION: 652..1518
 (D) OTHER INFORMATION: /codon_start= 652
 /product= "ATPase subunit"
 /gene= "atpG"
 /standard_name= "gamma subunit of the F1 portion
 of the FOF1 ATPase"
 /label= gamma-subunit

(ix) FEATURE:

(A) NAME/KEY: CDS
 (B) LOCATION: 1654..2205
 (D) OTHER INFORMATION: /partial
 /codon_start= 1654
 /product= "ATPase subunit, partial sequence"
 /gene= "atpD"
 /standard_name= "beta subunit of the F1 portion of
 the FOF1 ATPase"
 /label= beta-subunit

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 6:

TGA TTC TAC TTA CAT TCA CGT CTT TTG GAA CGT GCT GCC AAA TTA TCT	48
Phe Tyr Leu His Ser Arg Leu Leu Glu Arg Ala Ala Lys Leu Ser	
470 475 480	
GAC TAT CTT GGT GGT GGT TCA ATG ACT GCA CTG CCA TTC ATT GAA ACA	96
Asp Tyr Leu Gly Gly Gly Ser Met Thr Ala Leu Pro Phe Ile Glu Thr	
485 490 495 500	
CAA GCC GGA GAT ATC TCA GCT TAT ATT GCA ACA AAC GTT ATC TCT ATT	144
Gln Ala Gly Asp Ile Ser Ala Tyr Ile Ala Thr Asn Val Ile Ser Ile	
505 510 515	
ACT GAC GGT CAA ATT TTC CTT GAA AAT GAC TTA TTC TAT TCA GGT GTA	192
Thr Asp Gly Gln Ile Phe Leu Glu Asn Asp Leu Phe Tyr Ser Gly Val	
520 525 530	
CGT CCT GCC ATC GAT GCT GGT TCT TCA GTT TCT CGG GTT GGT GGT GCT	240
Arg Pro Ala Ile Asp Ala Gly Ser Ser Val Ser Arg Val Gly Gly Ala	
535 540 545	
GCA CAG ATC AAA GCC ATG AAG AAA GTT GCT GGT ACT TTG CGT CTT GAC	288
Ala Gln Ile Lys Ala Met Lys Lys Val Ala Gly Thr Leu Arg Leu Asp	
550 555 560	
CTT GCG TCA TTC CGT GAA CTT GAA GCC TTT ACT CAA TTT GGT TCT GAT	336
Leu Ala Ser Phe Arg Glu Leu Glu Ala Phe Thr Gln Phe Gly Ser Asp	
565 570 575 580	
CTT GAT GAA GCG ACT CAA GCA AAA TTG AAT CGT GGT CGT CGT ACC GTT	384
Leu Asp Glu Ala Thr Gln Ala Lys Leu Asn Arg Gly Arg Arg Thr Val	
585 590 595	
GAA GTT TTG AAG CAA CCA TTG CAC AAA CCA TTG GCT GTT GAA AAA CAA	432
Glu Val Leu Lys Gln Pro Leu His Lys Pro Leu Ala Val Glu Lys Gln	
600 605 610	
GTT TTA ATT CTT TAT GCA TTG ACT CAT GGT CAC TTG GAT GAT GTT CCA	480
Val Leu Ile Leu Tyr Ala Leu Thr His Gly His Leu Asp Asp Val Pro	
615 620 625	

GTT Val 630	GAT Asp 630	GAC Asp 630	GTC Val 630	CTT Leu 630	GAT Asp 635	TTT Phe 635	GAA Glu 635	ACA Thr 635	AAC Asn 635	AAT Asn 635	GTC Val 640	CGA Arg 640	TTC Phe 640	TTC Phe 640	GAT Asp 640	528
GCA Ala 645	AAT Asn 645	TAT Tyr 645	GCA Ala 645	AAA Lys 645	CTC Leu 650	TTG Leu 650	AAC Asn 655	GTG Val 655	ATT Ile 655	ACT Thr 655	GAA Glu 655	ACT Thr 655	AAA Lys 655	GAT Asp 660	TGC Cys 660	576
CAG Gln 665	AAG Lys 665	AAG Lys 665	CAA Gln 665	AAC Asn 665	TCG Ser 665	ACG Thr 665	AAG Lys 670	CAA Gln 670	TTA Leu 670	AAG Lys 670	CAT His 670	TCT Ser 670	AAA Lys 675	ATA Ile 675	CAA Gln 675	624
CGA Arg 1	ATT Ile 1	ATT Ile 1	AATAAGGAGG			CTAATCTA	ATG Met 1	GGA Gly 1	GCT Ala 1	TCA Ser 5	CTT Leu 5	AAT Asn 5	GAA Glu 5	ATA Ile 5		675
AAA Lys 10	ACT Thr 10	AAG Lys 10	ATT Ile 10	GCC Ala 10	TCA Ser 15	ACG Thr 15	AAG Lys 15	AAA Lys 15	ACA Thr 15	AGT Ser 20	CAA Gln 20	ATA Ile 20	ACT Thr 20	GGA Gly 20	GCC Ala 20	723
ATG Met 25	CAA Gln 25	ATG Met 25	GTT Val 25	TCC Ser 30	GCT Ala 30	GCG Ala 30	AAA Lys 30	CTT Leu 30	CAA Gln 35	AAA Lys 35	GCT Ala 35	GAA Glu 35	TCT Ser 35	CAT His 35	GCC Ala 40	771
AAA Lys 45	GCA Ala 45	TTT Phe 45	CAA Gln 45	ATT Ile 45	TAT Tyr 45	GCT Ala 45	GAA Glu 50	AAA Lys 50	GTT Val 50	CGT Arg 50	AAA Lys 50	ATT Ile 50	ACA Thr 55	ACT Thr 55	GAT Asp 55	819
TTA Leu 60	GTT Val 60	TCC Ser 60	TCT Ser 60	GAC Asp 60	AAA Lys 65	GAG Glu 65	CCA Pro 65	GCT Ala 65	AAG Lys 65	AAT Asn 65	CCA Pro 70	ATG Met 70	ATG Met 70	ATA Ile 70	GGA Gly 70	867
AGA Arg 75	GAA Glu 75	GTC Val 75	AAA Lys 75	AAA Lys 75	ACT Thr 80	GGC Gly 80	TAT Tyr 80	CTT Leu 80	GTA Val 80	ATT Ile 80	ACT Thr 85	TCG Ser 85	GAT Asp 85	CGT Arg 85	GGA Gly 85	915
CTT Leu 90	GTC Val 90	GGT Gly 90	GGC Gly 90	TAT Tyr 90	AAT Asn 95	TCA Ser 95	TAT Tyr 95	ATT Ile 95	TTG Leu 95	AAA Lys 100	TCT Ser 100	GTC Val 100	ATG Met 100	AAT Asn 100	ACT Thr 100	963
ATC Ile 105	CGT Arg 105	AAA Lys 105	CGT Arg 105	CCT Pro 110	GCT Ala 110	AAT Asn 110	GAA Glu 110	AGT Ser 110	GAA Glu 115	TAT Tyr 115	ACT Thr 115	ATT Ile 115	CTT Leu 115	GCA Ala 120	CTT Leu 120	1011
GGC Gly 125	GGT Gly 125	ACT Thr 125	GGA Gly 125	GCA Ala 125	GAT Asp 125	TTC Phe 130	TTC Phe 130	GGA Gly 130	GCA Ala 130	AGC Ser 130	AAT Asn 130	GTT Val 135	AAA Lys 135	AGT Ser 135	TTC Phe 135	1059
TTA Leu 140	GTC Val 140	CTT Leu 140	TGT Cys 140	GGT Gly 140	TTT Phe 145	TCA Ser 145	GAC Asp 145	CAA Gln 145	CCA Pro 145	AAT Asn 145	TTT Phe 150	GAA Glu 150	GAA Glu 150	GTT Val 150	AGA Arg 150	1107
GCG Ala 155	ATT Ile 155	GTT Val 155	ACA Thr 155	GAA Glu 155	GCG Ala 160	GTA Val 160	ACT Thr 160	GAA Glu 160	TAT Tyr 160	CAA Gln 165	GCA Ala 165	GAA Glu 165	GAA Glu 165	TTT Phe 165	GAT Asp 165	1155
GAA Glu 170	CTT Leu 170	TAT Tyr 170	GTT Val 170	TGC Cys 175	TAT Tyr 175	AAT Asn 175	CAC His 175	CAT His 175	GTG Val 180	AAC Asn 180	TCA Ser 180	TTG Leu 180	GTA Val 180	AGT Ser 180	GAA Glu 180	1203
GCA Ala 185	AGT Ser 185	ATG Met 185	GAA Glu 185	AAA Lys 190	ATG Met 190	TTG Leu 190	CCT Pro 190	ATT Ile 190	TTT Phe 195	TTT Phe 195	GAA Glu 195	GCA Ala 195	TCA Ser 195	GGT Gly 195	CAA Gln 200	1251

CAA AAA CCA TTT TTT GAA ACA TTT GAA TTA GAA CCA GAT TGT GAA ACA Gln Lys Pro Phe Phe Glu Thr Phe Glu Leu Glu Pro Asp Cys Glu Thr 205 210 215	1299
ATT TTA AAC CAA TTG TTG CCA CCA TAC GCT GAA AGT ATG ATT TAT GGT Ile Leu Asn Gln Leu Leu Pro Pro Tyr Ala Glu Ser Met Ile Tyr Gly 220 225 230	1347
TCA ATC GTT GAT GCT AAG ACA GCA GAA CAT GCT GCA GGT ATG ACA GCA Ser Ile Val Asp Ala Lys Thr Ala Glu His Ala Ala Gly Met Thr Ala 235 240 245	1395
ATG CGT ACT GCA ACT GAT AAT GCT CAC TCT GTT ATC AAT GAT TTG ACT Met Arg Thr Ala Thr Asp Asn Ala His Ser Val Ile Asn Asp Leu Thr 250 255 260	1443
ATT CAA TAC AAC CGT GCT CGT CAA GCA TCG ATT ACG CAA GAA ATT ACG Ile Gln Tyr Asn Arg Ala Arg Gln Ala Ser Ile Thr Gln Glu Ile Thr 265 270 275 280	1491
GAA ATC GTT GCA GGA GCC TCA GCG CTT TAATTTACTG ATAGGAATTC Glu Ile Val Ala Gly Ala Ser Ala Leu 285	1538
TGTCAGTGAT GGCTTTGAAT CTTAATTGTT TTTGTCAGTA AAATTTTAC TGACAAACAT	1598
AAAAATGAAT AGAAATTCTG TTCTTTGACA GAAAATAAAA ACAGGAGGAA AAACA TTG Leu 1	1656
AGT TCT GGT AAA ATT ACT CAG ATT ATC GGT CCC GTC GTT GAC GTG GAA Ser Ser Gly Lys Ile Thr Gln Ile Ile Gly Pro Val Val Asp Val Glu 5 10 15	1704
TTT GGT TCT GAT GCC AAA TTG CCT GAG ATT AAC AAT GCC TTG ATT GTC Phe Gly Ser Asp Ala Lys Leu Pro Glu Ile Asn Asn Ala Leu Ile Val 20 25 30	1752
TAC AAA GAT GTC AAT GGC CTA AAA ACA AAA ATT ACT CTT GAA GTT GCT Tyr Lys Asp Val Asn Gly Leu Lys Thr Lys Ile Thr Leu Glu Val Ala 35 40 45	1800
TTG GAA CTT GGT GAT GGT GCA GTT CGT ACA ATC GCT ATG GAA TCT ACT Leu Glu Leu Gly Asp Gly Ala Val Arg Thr Ile Ala Met Glu Ser Thr 50 55 60 65	1848
GAT GGC TTG ACT CGT GGA CTT GAA GTC CTT GAT ACA GGT AAA GCA GTC Asp Gly Leu Thr Arg Gly Leu Glu Val Leu Asp Thr Gly Lys Ala Val 70 75 80	1896
AGC GTT CCT GTT GGG GAA GCC ACT CTT GGT CGT GTT TTT AAC GTC CTT Ser Val Pro Val Gly Glu Ala Thr Leu Gly Arg Val Phe Asn Val Leu 85 90 95	1944
GGT GAT GTT ATT GAC GGT GGG GAA GAA TTT GCT GCT GAT GCA GAA CGT Gly Asp Val Ile Asp Gly Gly Glu Glu Phe Ala Ala Asp Ala Glu Arg 100 105 110	1992
AAT CCT ATC CAT AAA AAA GCT CCA ACA TTT GAC GAA TTG TCA ACT GCA Asn Pro Ile His Lys Lys Ala Pro Thr Phe Asp Glu Leu Ser Thr Ala 115 120 125	2040

AAC GAA GTT CTC GTA ACT GGG ATT AAA GTT GTC GAT TTG CTT GCA CCT	2088
Asn Glu Val Leu Val Thr Gly Ile Lys Val Val Asp Leu Leu Ala Pro	
130 135 140 145	
TAC CTT AAA GGT GGT AAA GTT GGA CTT TTC GGT GGT GCC GGA GTT GGT	2136
Tyr Leu Lys Gly Gly Lys Val Gly Leu Phe Gly Gly Ala Gly Val Gly	
150 155 160	
AAA GCC GTC CTT ATT CAA GAA TTG AAA CAC AAC ATC GCC CAA GAA CAC	2184
Lys Ala Val Leu Ile Gln Glu Leu Lys His Asn Ile Ala Gln Glu His	
165 170 175	
GGA GGT ATT TCT GTG TTT ACC GG	2207
Gly Gly Ile Ser Val Phe Thr	
180	

(2) INFORMATION FOR SEQ ID NO: 7:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 210 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 7:

Phe Tyr Leu His Ser Arg Leu Leu Glu Arg Ala Ala Lys Leu Ser Asp	
1 5 10 15	
Tyr Leu Gly Gly Gly Ser Met Thr Ala Leu Pro Phe Ile Glu Thr Gln	
20 25 30	
Ala Gly Asp Ile Ser Ala Tyr Ile Ala Thr Asn Val Ile Ser Ile Thr	
35 40 45	
Asp Gly Gln Ile Phe Leu Glu Asn Asp Leu Phe Tyr Ser Gly Val Arg	
50 55 60	
Pro Ala Ile Asp Ala Gly Ser Ser Val Ser Arg Val Gly Gly Ala Ala	
65 70 75 80	
Gln Ile Lys Ala Met Lys Lys Val Ala Gly Thr Leu Arg Leu Asp Leu	
85 90 95	
Ala Ser Phe Arg Glu Leu Glu Ala Phe Thr Gln Phe Gly Ser Asp Leu	
100 105 110	
Asp Glu Ala Thr Gln Ala Lys Leu Asn Arg Gly Arg Arg Thr Val Glu	
115 120 125	
Val Leu Lys Gln Pro Leu His Lys Pro Leu Ala Val Glu Lys Gln Val	
130 135 140	
Leu Ile Leu Tyr Ala Leu Thr His Gly His Leu Asp Asp Val Pro Val	
145 150 155 160	
Asp Asp Val Leu Asp Phe Glu Thr Asn Asn Val Arg Phe Phe Asp Ala	
165 170 175	
Asn Tyr Ala Lys Leu Leu Asn Val Ile Thr Glu Thr Lys Asp Cys Gln	
180 185 190	

Lys Lys Gln Asn Ser Thr Lys Gln Leu Lys His Ser Lys Ile Gln Arg
 195 200 205
 Ile Ile
 210

(2) INFORMATION FOR SEQ ID NO: 8:

(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 289 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 8:

Met Gly Ala Ser Leu Asn Glu Ile Lys Thr Lys Ile Ala Ser Thr Lys
 1 5 10 15
 Lys Thr Ser Gln Ile Thr Gly Ala Met Gln Met Val Ser Ala Ala Lys
 20 25 30
 Leu Gln Lys Ala Glu Ser His Ala Lys Ala Phe Gln Ile Tyr Ala Glu
 35 40 45
 Lys Val Arg Lys Ile Thr Thr Asp Leu Val Ser Ser Asp Lys Glu Pro
 50 55 60
 Ala Lys Asn Pro Met Met Ile Gly Arg Glu Val Lys Lys Thr Gly Tyr
 65 70 75 80
 Leu Val Ile Thr Ser Asp Arg Gly Leu Val Gly Gly Tyr Asn Ser Tyr
 85 90 95
 Ile Leu Lys Ser Val Met Asn Thr Ile Arg Lys Arg Pro Ala Asn Glu
 100 105 110
 Ser Glu Tyr Thr Ile Leu Ala Leu Gly Gly Thr Gly Ala Asp Phe Phe
 115 120 125
 Gly Ala Ser Asn Val Lys Ser Phe Leu Val Leu Cys Gly Phe Ser Asp
 130 135 140
 Gln Pro Asn Phe Glu Glu Val Arg Ala Ile Val Thr Glu Ala Val Thr
 145 150 155 160
 Glu Tyr Gln Ala Glu Glu Phe Asp Glu Leu Tyr Val Cys Tyr Asn His
 165 170 175
 His Val Asn Ser Leu Val Ser Glu Ala Ser Met Glu Lys Met Leu Pro
 180 185 190
 Ile Phe Phe Glu Ala Ser Gly Gln Gln Lys Pro Phe Phe Glu Thr Phe
 195 200 205
 Glu Leu Glu Pro Asp Cys Glu Thr Ile Leu Asn Gln Leu Leu Pro Pro
 210 215 220
 Tyr Ala Glu Ser Met Ile Tyr Gly Ser Ile Val Asp Ala Lys Thr Ala
 225 230 235 240

[illegible]

(2) INFORMATION FOR SEQ ID NO: 9:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 184 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 9:

Leu 1	Ser	Ser	Gly	Lys 5	Ile	Thr	Gln	Ile	Ile 10	Gly	Pro	Val	Val	Asp 15	Val
Glu	Phe	Gly	Ser 20	Asp	Ala	Lys	Leu	Pro 25	Glu	Ile	Asn	Asn	Ala 30	Leu	Ile
Val	Tyr	Lys 35	Asp	Val	Asn	Gly	Leu 40	Lys	Thr	Lys	Ile	Thr 45	Leu	Glu	Val
Ala	Leu 50	Glu	Leu	Gly	Asp	Gly 55	Ala	Val	Arg	Thr	Ile 60	Ala	Met	Glu	Ser
Thr 65	Asp	Gly	Leu	Thr	Arg 70	Gly	Leu	Glu	Val	Leu 75	Asp	Thr	Gly	Lys	Ala 80
Val	Ser	Val	Pro	Val 85	Gly	Glu	Ala	Thr	Leu 90	Gly	Arg	Val	Phe	Asn 95	Val
Leu	Gly	Asp	Val 100	Ile	Asp	Gly	Gly	Glu 105	Glu	Phe	Ala	Ala	Asp 110	Ala	Glu
Arg	Asn 115	Pro	Ile	His	Lys	Lys	Ala 120	Pro	Thr	Phe	Asp	Glu 125	Leu	Ser	Thr
Ala	Asn 130	Glu	Val	Leu	Val	Thr 135	Gly	Ile	Lys	Val	Val 140	Asp	Leu	Leu	Ala
Pro 145	Tyr	Leu	Lys	Gly 150	Gly	Lys	Val	Gly	Leu	Phe 155	Gly	Gly	Ala	Gly	Val 160
Gly	Lys	Ala	Val	Leu 165	Ile	Gln	Glu	Leu	Lys 170	His	Asn	Ile	Ala	Gln 175	Glu
His	Gly	Gly	Ile 180	Ser	Val	Phe	Thr								

(2) INFORMATION FOR SEQ ID NO: 10:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 2161 base pairs

(B) TYPE: nucleic acid
 (C) STRANDEDNESS: double
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Streptococcus thermophilus
 (B) STRAIN: ST3

(ix) FEATURE:

(A) NAME/KEY: CDS
 (B) LOCATION: 2..637
 (D) OTHER INFORMATION: /partial
 /codon_start= 2
 /product= "ATPase subunit, partial sequence"
 /gene= "atpA"
 /standard_name= "alpha subunit of the F1 portion
 of the F0F1 ATPase"
 /label= alpha-subunit

(ix) FEATURE:

(A) NAME/KEY: CDS
 (B) LOCATION: 659..1537
 (D) OTHER INFORMATION: /codon_start= 659
 /product= "ATPase subunit"
 /gene= "atpG"
 /standard_name= "gamma subunit of the F1 portion
 of the F0F1 ATPase"
 /label= gamma-subunit

(ix) FEATURE:

(A) NAME/KEY: CDS
 (B) LOCATION: 1616..2161
 (D) OTHER INFORMATION: /partial
 /codon_start= 1616
 /product= "ATPase subunit, partial sequence"
 /gene= "atpD"
 /standard_name= "beta subunit of the F1 portion of
 the F0F1 ATPase"
 /label= beta-subunit

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 10:

T	GAT	TCT	CAT	CTC	CAC	TCA	CGT	CTT	TTG	GAA	CGT	TCA	GCT	AAG	CTT	46
Asp	Ser	His	Leu	His	Ser	Arg	Leu	Leu	Glu	Arg	Ser	Ala	Lys	Leu		
185					190					195						
TCA	GAT	GAT	CTT	GGT	GGT	GGT	TCA	ATG	ACT	GCC	TTG	CCA	ATC	ATC	CAA	94
Ser	Asp	Asp	Leu	Gly	Gly	Gly	Ser	Met	Thr	Ala	Leu	Pro	Ile	Ile	Gln	
200				205					210					215		
ACA	CAA	GCA	GGA	GAT	ATC	TCA	GCT	TAT	ATC	GCG	ACA	AAC	GTT	ATT	TCT	142
Thr	Gln	Ala	Gly	Asp	Ile	Ser	Ala	Tyr	Ile	Ala	Thr	Asn	Val	Ile	Ser	
			220					225						230		

ATC	ACA	GAT	GGA	CAA	ATC	TTC	TTG	CAA	GAA	AAT	CTT	TTC	AAC	TCA	GGT	190
Ile	Thr	Asp	Gly	Gln	Ile	Phe	Leu	Gln	Glu	Asn	Leu	Phe	Asn	Ser	Gly	
			235					240					245			
ATT	CGT	CCT	GCG	ATT	GAT	GCT	GGT	TCT	TCA	GTA	TCA	CGT	GTT	GGT	GGT	238
Ile	Arg	Pro	Ala	Ile	Asp	Ala	Gly	Ser	Ser	Val	Ser	Arg	Val	Gly	Gly	
		250					255					260				
TCA	GCA	CAA	ATC	AAA	GCA	ATG	AAG	AAA	GTT	GCT	GGT	ACC	CTT	CGT	CTT	286
Ser	Ala	Gln	Ile	Lys	Ala	Met	Lys	Lys	Val	Ala	Gly	Thr	Leu	Arg	Leu	
	265					270					275					
GAC	TTG	GCT	TCT	CAC	CGT	GAA	CTT	GAA	GCC	TTT	ACA	CAA	TTC	GGT	TCT	334
Asp	Leu	Ala	Ser	His	Arg	Glu	Leu	Glu	Ala	Phe	Thr	Gln	Phe	Gly	Ser	
280					285					290					295	
GAT	TTG	GAT	GCC	GCA	ACA	CAA	GCT	AAA	CTT	AAT	CGT	GGA	CGT	CGT	ACA	382
Asp	Leu	Asp	Ala	Ala	Thr	Gln	Ala	Lys	Leu	Asn	Arg	Gly	Arg	Arg	Thr	
				300					305					310		
GTT	GAA	GTG	CTT	AAA	CAA	CCA	CTT	CAT	AAC	CCA	CTT	CCG	GTT	GAA	AAA	430
Val	Glu	Val	Leu	Lys	Gln	Pro	Leu	His	Asn	Pro	Leu	Pro	Val	Glu	Lys	
			315					320					325			
CAA	GTT	CTT	ATT	CTT	TAC	GCT	TTG	ACA	CAT	GGC	TTC	TTG	GAC	AGT	GTT	478
Gln	Val	Leu	Ile	Leu	Tyr	Ala	Leu	Thr	His	Gly	Phe	Leu	Asp	Ser	Val	
			330				335					340				
CCG	GTT	GAT	CAA	ATC	TTG	GAT	TTT	GAA	GAA	GCC	CTC	TAT	GAC	TAC	TTT	526
Pro	Val	Asp	Gln	Ile	Leu	Asp	Phe	Glu	Glu	Ala	Leu	Tyr	Asp	Tyr	Phe	
	345					350					355					
GAT	AGC	CAT	CAT	GAG	GAT	ATC	TTT	GAA	ACA	ATC	CGT	TCA	ACT	AAG	GAT	574
Asp	Ser	His	His	Glu	Asp	Ile	Phe	Glu	Thr	Ile	Arg	Ser	Thr	Lys	Asp	
360					365					370					375	
CTT	CCT	GAA	GAA	GCT	GTG	CTT	AAT	GAA	GCT	ATC	CAA	GCT	TTC	AAA	GAT	622
Leu	Pro	Glu	Glu	Ala	Val	Leu	Asn	Glu	Ala	Ile	Gln	Ala	Phe	Lys	Asp	
				380				385						390		
CAA	TCG	GAA	TAC	AAA	TAGAGATAGG	GAGGACAGCA	T	ATG	GCA	GGC	TCT	CTA				673
Gln	Ser	Glu	Tyr	Lys				Met	Ala	Gly	Ser	Leu				
			395					1				5				
AGA	GAA	ATC	AAA	GCA	AAA	ATT	GCT	TCA	ATT	AAG	CAA	ACG	AGT	CAT	ATT	721
Arg	Glu	Ile	Lys	Ala	Lys	Ile	Ala	Ser	Ile	Lys	Gln	Thr	Ser	His	Ile	
				10					15					20		
ACA	GGA	GCC	ATG	CAA	ATG	GTT	TCT	GCT	TCT	AAA	TTG	ACA	CGT	TCT	GAG	769
Thr	Gly	Ala	Met	Gln	Met	Val	Ser	Ala	Ser	Lys	Leu	Thr	Arg	Ser	Glu	
			25					30					35			
CAG	GCT	GCT	AAA	GAT	TTC	CAA	ATC	TAT	GCC	TCA	AAA	ATT	AGA	CAG	ATC	817
Gln	Ala	Ala	Lys	Asp	Phe	Gln	Ile	Tyr	Ala	Ser	Lys	Ile	Arg	Gln	Ile	
			40				45					50				
ACA	ACA	GAT	CTT	CTA	CAT	TCA	GAA	TTG	GTT	AAT	GGT	TCT	TCA	AAT	CCG	865
Thr	Thr	Asp	Leu	Leu	His	Ser	Glu	Leu	Val	Asn	Gly	Ser	Ser	Asn	Pro	
	55					60					65					
ATG	TTG	GAT	GCA	CGT	CCA	GTT	CGT	AAG	TCA	GGG	TAT	ATT	GTC	ATT	ACT	913
Met	Leu	Asp	Ala	Arg	Pro	Val	Arg	Lys	Ser	Gly	Tyr	Ile	Val	Ile	Thr	
70					75					80					85	

TCA GAT AAG GGA TTA GTT GGA GGA TAT AAT TCA ACC ATT CTT AAA GCT	961
Ser Asp Lys Gly Leu Val Gly Gly Tyr Asn Ser Thr Ile Leu Lys Ala	
90 95 100	
GTC TTG GAT ATG ATT AAA CGT GAC CAT GAT TCT GAA GAT GAA TAT GCT	1009
Val Leu Asp Met Ile Lys Arg Asp His Asp Ser Glu Asp Glu Tyr Ala	
105 110 115	
ATC ATC TCT ATT GGT GGA ACA GGT TCA GAT TTC TTC AAA GCT CGT AAC	1057
Ile Ile Ser Ile Gly Gly Thr Gly Ser Asp Phe Phe Lys Ala Arg Asn	
120 125 130	
ATG AAT GTT GCT TTT GAA CTT CGT GGC CTT GAA GAT CAA CCT AGT TTC	1105
Met Asn Val Ala Phe Glu Leu Arg Gly Leu Glu Asp Gln Pro Ser Phe	
135 140 145	
GAT CAA GTC GGG GAA ATC ATT CTA AAA GCT GTA GGA ATG TAT CAA AAT	1153
Asp Gln Val Gly Glu Ile Leu Lys Ala Val Gly Met Tyr Gln Asn	
150 155 160 165	
GAG CTT TTT GAT GAA CTT TAT GTG TGT TAC AAT CAT CAT ATT AAT AGT	1201
Glu Leu Phe Asp Glu Leu Tyr Val Cys Tyr Asn His His Ile Asn Ser	
170 175 180	
TTG TTT TGT GAA GTT TGT GTT GAA AAA ATG CTT CCA ATT GCT GAT TTT	1249
Leu Phe Cys Glu Val Cys Val Glu Lys Met Leu Pro Ile Ala Asp Phe	
185 190 195	
GAT CCT AAT GAA TTT GAA GGC CAT GTA TTG ACC AAG TTT GAA TTG GAA	1297
Asp Pro Asn Glu Phe Glu Gly His Val Leu Thr Lys Phe Glu Leu Glu	
200 205 210	
CCA AGT TGT GAT ACT ATT TTG GAT CAA CTT TTG CCC ACA ATA GTC GGT	1345
Pro Ser Cys Asp Thr Ile Leu Asp Gln Leu Leu Pro Thr Ile Val Gly	
215 220 225	
GAG AGT TTT ATC TAC GGT GCT ATC GTA GAT GCC AAA ACA GCT GAG CAT	1393
Glu Ser Phe Ile Tyr Gly Ala Ile Val Asp Ala Lys Thr Ala Glu His	
230 235 240 245	
GCT GCT GGT ATG ACC GCA ATG CAG ACT GCC ACT GAT AAT GCT AAG AAA	1441
Ala Ala Gly Met Thr Ala Met Gln Thr Ala Thr Asp Asn Ala Lys Lys	
250 255 260	
ATA ATT AAC GAT TTA ACA ATT CAA TAC AAC CGT GCA CGT CAA GCA GCC	1489
Ile Ile Asn Asp Leu Thr Ile Gln Tyr Asn Arg Ala Arg Gln Ala Ala	
265 270 275	
ATT ACT CAG GAA ATC ACT GAG ATT GTT GGC GGT GCT AGT GCA CTT GAA	1537
Ile Thr Gln Glu Ile Thr Glu Ile Val Gly Gly Ala Ser Ala Leu Glu	
280 285 290	
TAGCTAGAGA TTTGTCTTGA TTTGACATAC AATAAAAAGG GATGATTGTC ATCCAGAAAA	1597
CTTCATAAGG AGAAAAACA ATG AGC TCA GGC AAA ATT GCT CAG GTT GTT GGT	1648
Met Ser Ser Gly Lys Ile Ala Gln Val Val Gly	
1 5 10	
CCT GTT GTA GAC GTA GCG TTT GCA ACT GGC GAT AAA CTT CCT GAG ATT	1696
Pro Val Val Asp Val Ala Phe Ala Thr Gly Asp Lys Leu Pro Glu Ile	
15 20 25	

AAC AAT GCA TTG GTC GTT TAC ACT GAG AAG AAA AGT CTT AGA CGG ATG	1744
Asn Asn Ala Leu Val Val Tyr Thr Glu Lys Lys Ser Leu Arg Arg Met	
30 35 40	
GTG CTC GAA GTA GCT TCG TTG AAA CTT GGA GAA GGT GTG GTT CGT ACC	1792
Val Leu Glu Val Ala Ser Leu Lys Leu Gly Glu Gly Val Val Arg Thr	
45 50 55	
ATT GCC ATG GAA TCT ACT GAT GGA TTG ACT CGT GGG CTA GAA GTT CTG	1840
Ile Ala Met Glu Ser Thr Asp Gly Leu Thr Arg Gly Leu Glu Val Leu	
60 65 70 75	
GAC ACA GGT CGT CCA ATC AGT GTT CCT GTT GGT AAA GAA CTT CTT GGA	1888
Asp Thr Gly Arg Pro Ile Ser Val Pro Val Gly Lys Glu Leu Leu Gly	
80 85 90	
CGT GTC TTT AAC GTG CTT GGA GAT ACC ATT GAC ATG GAA GCA CCT TTT	1936
Arg Val Phe Asn Val Leu Gly Asp Thr Ile Asp Met Glu Ala Pro Phe	
95 100 105	
GCA GAT GAT GCA GAG CGT GAA CCA ATT CAT AAA AAA GCA CCT ACC TTC	1984
Ala Asp Asp Ala Glu Arg Glu Pro Ile His Lys Lys Ala Pro Thr Phe	
110 115 120	
GAT GAA TTG TCA ACA AGT ACT GAA ATC CTT GAA ACA GGG ATT AAA GTT	2032
Asp Glu Leu Ser Thr Ser Thr Glu Ile Leu Glu Thr Gly Ile Lys Val	
125 130 135	
ATC GAC TTG CTT GCC CCT TAT CTT AAA GGT GGT AAA GTC GGA CTT TTC	2080
Ile Asp Leu Leu Ala Pro Tyr Leu Lys Gly Gly Lys Val Gly Leu Phe	
140 145 150 155	
GGT GGT GCC GGT GTT GGT AAG GCC GTT CTT ATT CAA GAG CTG AAT CAC	2128
Gly Gly Ala Gly Val Gly Lys Ala Val Leu Ile Gln Glu Leu Asn His	
160 165 170	
AAC ATT GCT CAA GAA CAC GGT GGC ATT TCC GTG	2161
Asn Ile Ala Gln Glu His Gly Gly Ile Ser Val	
175 180	

(2) INFORMATION FOR SEQ ID NO: 11:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 212 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 11:

Asp Ser His Leu His Ser Arg Leu Leu Glu Arg Ser Ala Lys Leu Ser
1 5 10 15
Asp Asp Leu Gly Gly Gly Ser Met Thr Ala Leu Pro Ile Ile Gln Thr
20 25 30
Gln Ala Gly Asp Ile Ser Ala Tyr Ile Ala Thr Asn Val Ile Ser Ile
35 40 45
Thr Asp Gly Gln Ile Phe Leu Gln Glu Asn Leu Phe Asn Ser Gly Ile
50 55 60

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Arg Pro Ala Ile Asp Ala Gly Ser Ser Val Ser Arg Val Gly Gly Ser
65              70              75              80

Ala Gln Ile Lys Ala Met Lys Lys Val Ala Gly Thr Leu Arg Leu Asp
85              90              95

Leu Ala Ser His Arg Glu Leu Glu Ala Phe Thr Gln Phe Gly Ser Asp
100            105            110

Leu Asp Ala Ala Thr Gln Ala Lys Leu Asn Arg Gly Arg Arg Thr Val
115            120            125

Glu Val Leu Lys Gln Pro Leu His Asn Pro Leu Pro Val Glu Lys Gln
130            135            140

Val Leu Ile Leu Tyr Ala Leu Thr His Gly Phe Leu Asp Ser Val Pro
145            150            155            160

Val Asp Gln Ile Leu Asp Phe Glu Glu Ala Leu Tyr Asp Tyr Phe Asp
165            170            175

Ser His His Glu Asp Ile Phe Glu Thr Ile Arg Ser Thr Lys Asp Leu
180            185            190

Pro Glu Glu Ala Val Leu Asn Glu Ala Ile Gln Ala Phe Lys Asp Gln
195            200            205

Ser Glu Tyr Lys
210

```

(2) INFORMATION FOR SEQ ID NO: 12:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 293 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 12:

```

Met Ala Gly Ser Leu Arg Glu Ile Lys Ala Lys Ile Ala Ser Ile Lys
1              5              10              15

Gln Thr Ser His Ile Thr Gly Ala Met Gln Met Val Ser Ala Ser Lys
20            25            30

Leu Thr Arg Ser Glu Gln Ala Ala Lys Asp Phe Gln Ile Tyr Ala Ser
35            40            45

Lys Ile Arg Gln Ile Thr Thr Asp Leu Leu His Ser Glu Leu Val Asn
50            55            60

Gly Ser Ser Asn Pro Met Leu Asp Ala Arg Pro Val Arg Lys Ser Gly
65            70            75            80

Tyr Ile Val Ile Thr Ser Asp Lys Gly Leu Val Gly Gly Tyr Asn Ser
85            90            95

Thr Ile Leu Lys Ala Val Leu Asp Met Ile Lys Arg Asp His Asp Ser
100           105           110

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Glu Asp Glu Tyr Ala Ile Ile Ser Ile Gly Gly Thr Gly Ser Asp Phe
 115 120 125
 Phe Lys Ala Arg Asn Met Asn Val Ala Phe Glu Leu Arg Gly Leu Glu
 130 135 140
 Asp Gln Pro Ser Phe Asp Gln Val Gly Glu Ile Ile Leu Lys Ala Val
 145 150 155 160
 Gly Met Tyr Gln Asn Glu Leu Phe Asp Glu Leu Tyr Val Cys Tyr Asn
 165 170 175
 His His Ile Asn Ser Leu Phe Cys Glu Val Cys Val Glu Lys Met Leu
 180 185 190
 Pro Ile Ala Asp Phe Asp Pro Asn Glu Phe Glu Gly His Val Leu Thr
 195 200 205
 Lys Phe Glu Leu Glu Pro Ser Cys Asp Thr Ile Leu Asp Gln Leu Leu
 210 215 220
 Pro Thr Ile Val Gly Glu Ser Phe Ile Tyr Gly Ala Ile Val Asp Ala
 225 230 235 240
 Lys Thr Ala Glu His Ala Ala Gly Met Thr Ala Met Gln Thr Ala Thr
 245 250 255
 Asp Asn Ala Lys Lys Ile Ile Asn Asp Leu Thr Ile Gln Tyr Asn Arg
 260 265 270
 Ala Arg Gln Ala Ala Ile Thr Gln Glu Ile Thr Glu Ile Val Gly Gly
 275 280 285
 Ala Ser Ala Leu Glu
 290

(2) INFORMATION FOR SEQ ID NO: 13:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 182 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 13:

Met Ser Ser Gly Lys Ile Ala Gln Val Val Gly Pro Val Val Asp Val
 1 5 10 15
 Ala Phe Ala Thr Gly Asp Lys Leu Pro Glu Ile Asn Asn Ala Leu Val
 20 25 30
 Val Tyr Thr Glu Lys Lys Ser Leu Arg Arg Met Val Leu Glu Val Ala
 35 40 45
 Ser Leu Lys Leu Gly Glu Gly Val Val Arg Thr Ile Ala Met Glu Ser
 50 55 60
 Thr Asp Gly Leu Thr Arg Gly Leu Glu Val Leu Asp Thr Gly Arg Pro
 65 70 75 80

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Ile Ser Val Pro Val Gly Lys Glu Leu Leu Gly Arg Val Phe Asn Val
      85                      90                      95
Leu Gly Asp Thr Ile Asp Met Glu Ala Pro Phe Ala Asp Asp Ala Glu
      100                      105                      110
Arg Glu Pro Ile His Lys Lys Ala Pro Thr Phe Asp Glu Leu Ser Thr
      115                      120                      125
Ser Thr Glu Ile Leu Glu Thr Gly Ile Lys Val Ile Asp Leu Leu Ala
      130                      135                      140
Pro Tyr Leu Lys Gly Gly Lys Val Gly Leu Phe Gly Gly Ala Gly Val
      145                      150                      155                      160
Gly Lys Ala Val Leu Ile Gln Glu Leu Asn His Asn Ile Ala Gln Glu
      165                      170                      175
His Gly Gly Ile Ser Val
      180

```

(2) INFORMATION FOR SEQ ID NO: 14:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 914 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(v) FRAGMENT TYPE: C-terminal

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: *Phaffia rhodozyma*

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 51..824
- (D) OTHER INFORMATION: /partial
 - /codon_start= 51
 - /product= "ATPase subunit, partial sequence"
 - /gene= "ATP2"
 - /standard_name= "beta subunit of the F1 portion of the FOF1 ATPase"
 - /label= beta-subunit

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 14:

```

GAATTCTCAA CCTGAGGGT GACTCCAAGG TCGCTCTTGT CTTCGGACAG ATG AAC      56
                               Met Asn

GAG CCC CCG GGT GCT CGA GCC CGA GTC GCT TTG ACT GGT TTG ACC ATC      104
Glu Pro Pro Gly Ala Arg Ala Arg Val Ala Leu Thr Gly Leu Thr Ile
185                      190                      195                      200

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GCC GAG TAC TTC CGA GAC GAG GAA GGA CAG GAT GTC TTG CTT TTC ATC	152
Ala Glu Tyr Phe Arg Asp Glu Glu Gly Gln Asp Val Leu Leu Phe Ile	
205 210 215	
GAC AAC ATT TTC CGA TTC ACC CAG GCC GGT TCT GAG GTG TCT GCC TTG	200
Asp Asn Ile Phe Arg Phe Thr Gln Ala Gly Ser Glu Val Ser Ala Leu	
220 225 230	
CTT GGT CGA ATT CCC TCC GCC GTC GGA TAC CAG CCC ACT CTT TCC ACC	248
Leu Gly Arg Ile Pro Ser Ala Val Gly Tyr Gln Pro Thr Leu Ser Thr	
235 240 245	
GAT ATG GGA GGT ATG CAG GAG CGA ATT ACC ACC ACC AAG AAG GGA TCC	296
Asp Met Gly Gly Met Gln Glu Arg Ile Thr Thr Thr Lys Lys Gly Ser	
250 255 260	
ATC ACT TCC GTC CAG GCC GTC TAC GTG CCT GCT GAT GAT TTG ACC GAT	344
Ile Thr Ser Val Gln Ala Val Tyr Val Pro Ala Asp Asp Leu Thr Asp	
265 270 275 280	
CCT GCC CCC GCC ACC ACC TTC GCC CAC TTG GAC GCC ACC ACT GTG TTG	392
Pro Ala Pro Ala Thr Thr Phe Ala His Leu Asp Ala Thr Thr Val Leu	
285 290 295	
TCT CGA GGT ATC GCT GAG TTG GGT ATC TAC CCC GCT GTC GAT CCC CTT	440
Ser Arg Gly Ile Ala Glu Leu Gly Ile Tyr Pro Ala Val Asp Pro Leu	
300 305 310	
GAT TCT AAG TCC CGA ATG CTC GAC CCC CGA ATT GTC GGA CAG GAG CAC	488
Asp Ser Lys Ser Arg Met Leu Asp Pro Arg Ile Val Gly Gln Glu His	
315 320 325	
TAC GAC ATC GCC ACC AAG ACC CAG AAG ATC CTC CAG GAC TAC AAG TCC	536
Tyr Asp Ile Ala Thr Lys Thr Gln Lys Ile Leu Gln Asp Tyr Lys Ser	
330 335 340	
CTC CAG GAT ATC ATT GCC ATT CTT GGT ATG GAT GAG TTG TCT GAG GAG	584
Leu Gln Asp Ile Ile Ala Ile Leu Gly Met Asp Glu Leu Ser Glu Glu	
345 350 355 360	
GAC AAG TTG ACC GTC GAG CGA GCC CGA AAG ATC CAG CGA TTC ATG TCG	632
Asp Lys Leu Thr Val Glu Arg Ala Arg Lys Ile Gln Arg Phe Met Ser	
365 370 375	
CAG CCT TTC GCT GTC GCT CAG GTC TTC ACT GGT ATC GAG GGA AAG CTT	680
Gln Pro Phe Ala Val Ala Gln Val Phe Thr Gly Ile Glu Gly Lys Leu	
380 385 390	
GTT CCC TTG AAG ACT ACT TTG GAG TCC TTT AAG GAG CTT CTT TCC GGA	728
Val Pro Leu Lys Thr Thr Leu Glu Ser Phe Lys Glu Leu Leu Ser Gly	
395 400 405	
GCC TGC GAC CAC CTC CCT GAG TCT GCT TTC TAC ATG GTT GGT GAC ATC	776
Ala Cys Asp His Leu Pro Glu Ser Ala Phe Tyr Met Val Gly Asp Ile	
410 415 420	
GCT GAT GTC AAG GCC AAG GCT GCT GCC CAG GCT AAG GAG TTG GCT GCT	824
Ala Asp Val Lys Ala Lys Ala Ala Ala Gln Ala Lys Glu Leu Ala Ala	
425 430 435 440	
TAAGAGAAGA GTTGTCGAAT GTGTTTCGAG GTGTCAGAGT TGTCTTTTAT GAATGTTTCT	884
ATCTCCTTAA AAAAAAAAAA AAAAAAAAAA	914

(2) INFORMATION FOR SEQ ID NO: 15:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 258 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 15:

```

Met Asn Glu Pro Pro Gly Ala Arg Ala Arg Val Ala Leu Thr Gly Leu
 1           5           10           15
Thr Ile Ala Glu Tyr Phe Arg Asp Glu Glu Gly Gln Asp Val Leu Leu
          20           25           30
Phe Ile Asp Asn Ile Phe Arg Phe Thr Gln Ala Gly Ser Glu Val Ser
          35           40           45
Ala Leu Leu Gly Arg Ile Pro Ser Ala Val Gly Tyr Gln Pro Thr Leu
          50           55           60
Ser Thr Asp Met Gly Gly Met Gln Glu Arg Ile Thr Thr Thr Lys Lys
          65           70           75           80
Gly Ser Ile Thr Ser Val Gln Ala Val Tyr Val Pro Ala Asp Asp Leu
          85           90           95
Thr Asp Pro Ala Pro Ala Thr Thr Phe Ala His Leu Asp Ala Thr Thr
          100          105          110
Val Leu Ser Arg Gly Ile Ala Glu Leu Gly Ile Tyr Pro Ala Val Asp
          115          120          125
Pro Leu Asp Ser Lys Ser Arg Met Leu Asp Pro Arg Ile Val Gly Gln
          130          135          140
Glu His Tyr Asp Ile Ala Thr Lys Thr Gln Lys Ile Leu Gln Asp Tyr
          145          150          155          160
Lys Ser Leu Gln Asp Ile Ile Ala Ile Leu Gly Met Asp Glu Leu Ser
          165          170          175
Glu Glu Asp Lys Leu Thr Val Glu Arg Ala Arg Lys Ile Gln Arg Phe
          180          185          190
Met Ser Gln Pro Phe Ala Val Ala Gln Val Phe Thr Gly Ile Glu Gly
          195          200          205
Lys Leu Val Pro Leu Lys Thr Thr Leu Glu Ser Phe Lys Glu Leu Leu
          210          215          220
Ser Gly Ala Cys Asp His Leu Pro Glu Ser Ala Phe Tyr Met Val Gly
          225          230          235          240
Asp Ile Ala Asp Val Lys Ala Lys Ala Ala Ala Gln Ala Lys Glu Leu
          245          250          255
Ala Ala

```

(2) INFORMATION FOR SEQ ID NO: 16:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 375 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: *Trichoderma reesei*

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 50..361
- (D) OTHER INFORMATION: /partial
 /codon_start= 50
 /product= "ATPase subunit, partial sequence"
 /gene= "ATP2"
 /standard_name= "beta subunit of F1 portion of the FOF1 ATPase"
 /label= beta-subunit

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 16:

TACTCGAAGA ATTCGGCACG AGGCTGATTG CTCTCGGTCA TCTGCCAAG ATG TTC	55
Met Phe	
260	
AAG AGC GGC GTT TCG TCC CTC GCC AGG GCT GCC CGC CCA TCA ATT ACC	103
Lys Ser Gly Val Ser Ser Leu Ala Arg Ala Ala Arg Pro Ser Ile Thr	
265 270 275	
GCT CGA CGA GCT ATC CGA CCA GCC TTC CCT CGA ACC CCC CTC GCG AGG	151
Ala Arg Arg Ala Ile Arg Pro Ala Phe Pro Arg Thr Pro Leu Ala Arg	
280 285 290	
CTT GCC AGC ACC CAG AGC GTC GGA GAT GGC AAG ATC CAC CAG GTC ATT	199
Leu Ala Ser Thr Gln Ser Val Gly Asp Gly Lys Ile His Gln Val Ile	
295 300 305	
GGT GCC GTC GTC GAC GTC AAG TTC GAC ACC GCC AAG CTG CCT CCT ATC	247
Gly Ala Val Val Asp Val Lys Phe Asp Thr Ala Lys Leu Pro Pro Ile	
310 315 320	
CTG AAC GCC CTG GAG ACC ACC AAC AAC AAC CAG AAG CTG GTC CTC GAG	295
Leu Asn Ala Leu Glu Thr Thr Asn Asn Asn Gln Lys Leu Val Leu Glu	
325 330 335 340	
GTG GCT CAA CAC TTG GGC GAG AAT GTC GTT CGC TGC ATT GCC ATG GAC	343
Val Ala Gln His Leu Gly Glu Asn Val Val Arg Cys Ile Ala Met Asp	
345 350 355	
GGA TCC GAG GGT CTC GTC GTGGTTCCAA GGCA	375
Gly Ser Glu Gly Leu Val	
360	

(2) INFORMATION FOR SEQ ID NO: 17:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 104 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 17:

```
Met Phe Lys Ser Gly Val Ser Ser Leu Ala Arg Ala Ala Arg Pro Ser
 1           5           10           15
Ile Thr Ala Arg Arg Ala Ile Arg Pro Ala Phe Pro Arg Thr Pro Leu
          20           25           30
Ala Arg Leu Ala Ser Thr Gln Ser Val Gly Asp Gly Lys Ile His Gln
          35           40           45
Val Ile Gly Ala Val Val Asp Val Lys Phe Asp Thr Ala Lys Leu Pro
          50           55           60
Pro Ile Leu Asn Ala Leu Glu Thr Thr Asn Asn Asn Gln Lys Leu Val
          65           70           75           80
Leu Glu Val Ala Gln His Leu Gly Glu Asn Val Val Arg Cys Ile Ala
          85           90           95
Met Asp Gly Ser Glu Gly Leu Val
          100
```

PATENT CLAIMS

1. A method of improving the production of biomass or a desired product from a cell, characterized by expressing
5 an uncoupled ATPase activity in said cell to induce conversion of ATP to ADP without primary effects on other cellular metabolites or functions, and incubating the cell with a suitable substrate to produce said biomass or product.
- 10 2. A method according to claim 1, characterized by expressing in said cell the soluble part (F₁) of the membrane bound (F₀F₁ type) H⁺-ATPase or a portion of F₁ exhibiting ATPase activity.
- 15 3. A method according to claim 1 or 2, wherein said cell is a prokaryotic cell.
- 20 4. A method according to claim 3, wherein said cell is selected from the group consisting of bacteria belonging to the genera *Lactococcus*, *Streptococcus*, *Enterococcus*, *Lactobacillus*, *Leuconostoc*, *Escherichia*, *Zymomonas*, *Bacillus* and *Pseudomonas*.
- 25 5. A method according to claim 1 or 2, wherein said cell is a eukaryotic cell.
6. A method according to claim 5, wherein said cell is a yeast cell.
- 30 7. A method according to claim 6, wherein said cell belongs to *Saccharomyces cerevisiae* or *Trichoderma reesei*.
- 35 8. A method according to any one of claims 1-7, wherein said cell is transformed or transfected with an expression vector including DNA encoding F₁ or a portion thereof exhibiting ATPase activity under the control of a

promoter functioning in said cell, and said DNA is expressed in the cell.

9. A method according to claim 8, wherein said DNA encoding F₁ or a portion thereof is homologous to said cell.

10. A method according to claim 8, wherein said DNA encoding F₁ or a portion thereof is heterologous to said cell.

11. A method according to any one of claims 8-10, wherein said DNA encoding F₁ or a portion thereof is derived from a prokaryotic organism.

12. A method according to claim 11, wherein said DNA encoding F₁ or a portion thereof is derived from *Escherichia coli*, *Lactococcus lactis* or *Streptococcus thermophilus* and is selected from the group consisting of the gene encoding the F₁ subunit β or a portion thereof and various combinations of said gene or portion with the genes encoding the F₁ subunits δ , α , γ and ϵ or portions thereof.

13. A method according to claim 12, wherein said DNA encoding F₁ or a portion thereof is selected from the group consisting of the *Escherichia coli*, *Streptococcus thermophilus* and *Lactococcus lactis* genes *atpHAGDC* (coding for subunits δ , α , γ , β , ϵ), *atpAGDC* (coding for subunits α , γ , β , ϵ), *atpAGD* (coding for subunits α , γ , β), *atpDC* (coding for subunits β , ϵ) and *atpD* (coding for subunit β alone).

14. A method according to any one of claims 8-10, wherein said DNA encoding F₁ or a portion thereof is derived from a eukaryotic organism.

15. A method according to claim 14, wherein said DNA encoding F₁ or a portion thereof is derived from *Saccharomyces cerevisiae*, *Phaffia rhodozyma* or *Trichoderma reesei* and is selected from the group consisting of the gene encoding the F₁ subunit β or a portion thereof and various combinations of said gene or portion with the genes encoding the other F₁ subunits or portions thereof.

16. A vector including DNA encoding the soluble part (F₁) of the membrane bound (F₀F₁ type) H⁺-ATPase or a portion of F₁ exhibiting ATPase activity, said DNA being derived from *Lactococcus lactis* subsp. *cremoris* and having the sequence stated in SEQ ID No. 1.

17. A vector including DNA encoding the soluble part (F₁) of the membrane bound (F₀F₁ type) H⁺-ATPase or a portion of F₁ exhibiting ATPase activity, said DNA being derived from *Lactococcus lactis* subsp. *lactis* and having the sequence stated in SEQ ID No. 6.

18. A vector including DNA encoding the soluble part (F₁) of the membrane bound (F₀F₁ type) H⁺-ATPase or a portion of F₁ exhibiting ATPase activity, said DNA being derived from *Streptococcus thermophilus* and having the sequence stated in SEQ ID No. 10.

19. A vector including DNA encoding the soluble part (F₁) of the membrane bound (F₀F₁ type) H⁺-ATPase or a portion of F₁ exhibiting ATPase activity, said DNA being derived from *Phaffia rhodozyma* and having the sequence stated in SEQ ID No. 14.

20. A vector including DNA encoding the soluble part (F₁) of the membrane bound (F₀F₁ type) H⁺-ATPase or a portion of F₁ exhibiting ATPase activity, said DNA being derived from *Trichoderma reesei* and having the sequence stated in SEQ ID No. 16.

21. An expression vector including DNA as defined in any one of claims 16-20 under the control of a promoter capable of directing the expression of said DNA in a prokaryotic or eukaryotic cell.

5

22. A method of optimizing the formation of biomass or a desired product by a cell, characterized by expressing different levels of uncoupled ATPase activity in the cell, incubating the cell on a suitable substrate, measuring the conversion rate of substrate into biomass or the desired product at each level of ATPase expression, and choosing a level of ATPase expression at which the conversion rate is optimized.

15 23. A method according to claim 22, wherein a number of specimens of said cell are transformed or transfected with their respective expression vector each including DNA encoding a different portion of the cytoplasmic part (F₁) of the membrane bound (F₀F₁ type) H⁺-ATPase up to and including the entire F₁, each portion exhibiting ATPase activity, said DNA in each expression vector being under the control of a promoter functioning in said cell, incubating each cell specimen on a suitable substrate, measuring the conversion rate of substrate into biomass or the desired product by each specimen, and choosing a specimen yielding an optimized conversion rate.

24. A method according to claim 22, wherein a number of specimens of said cell are transformed or transfected with their respective expression vector including DNA encoding a portion of the cytoplasmic part (F₁) of the membrane bound (F₀F₁ type) H⁺-ATPase up to and including the entire F₁, said portion exhibiting ATPase activity, said DNA in the respective expression vectors being under the control of each of a series of promoters covering a broad range of promoter activities and functioning in said cell, incubating each cell specimen on a suitable sub-

strate, measuring the conversion rate of substrate into biomass or the desired product by each specimen, and choosing a specimen yielding an optimized conversion rate.

5

25. A method according to claim 24, wherein the respective expression vectors include DNA encoding different such portions of F₁ up to and including the entire F₁, each DNA in respective expression vectors being under the control of each of a series of promoters covering a broad range of promoter activities and functioning in said cell.

26. A method according to any one of claims 23-25, wherein the promoter in each expression vector is an inducible promoter, and each cell specimen is grown at different concentrations of inducer.

27. A method according to any one of claims 23-26, wherein said DNA encoding a portion of F₁ up to and including the entire F₁ is homologous to said cell.

28. A method according to any one of claims 23-26, wherein said DNA encoding a portion of F₁ up to and including the entire F₁ is heterologous to said cell.

29. A method according to any one of claims 23-28, wherein said DNA encoding a portion of F₁ up to and including the entire F₁ is derived from a prokaryotic organism.

30. A method according to claim 29, wherein said DNA encoding a portion of F₁ up to and including the entire F₁ is derived from *Escherichia coli*, *Lactococcus lactis* or *Streptococcus thermophilus* and is selected from the group consisting of the gene encoding the F₁ subunit β or a portion thereof and various combinations of said gene or

portion with the genes encoding the F₁ subunits δ , α , γ and ϵ or portions thereof.

31. A method according to claim 30, wherein said DNA encoding a portion of F₁ up to and including the entire F₁ is selected from the group consisting of the *E. coli* genes *atpAGDC* (coding for subunits α , γ , β , ϵ), *atpAGD* (coding for subunits α , γ , β), *atpDC* (coding for subunits β , ϵ) and *atpD* (coding for subunit β alone).

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32. A method according to any one of claims 23-28, wherein said DNA encoding a portion of F₁ up to and including the entire F₁ is derived from a eukaryotic organism.

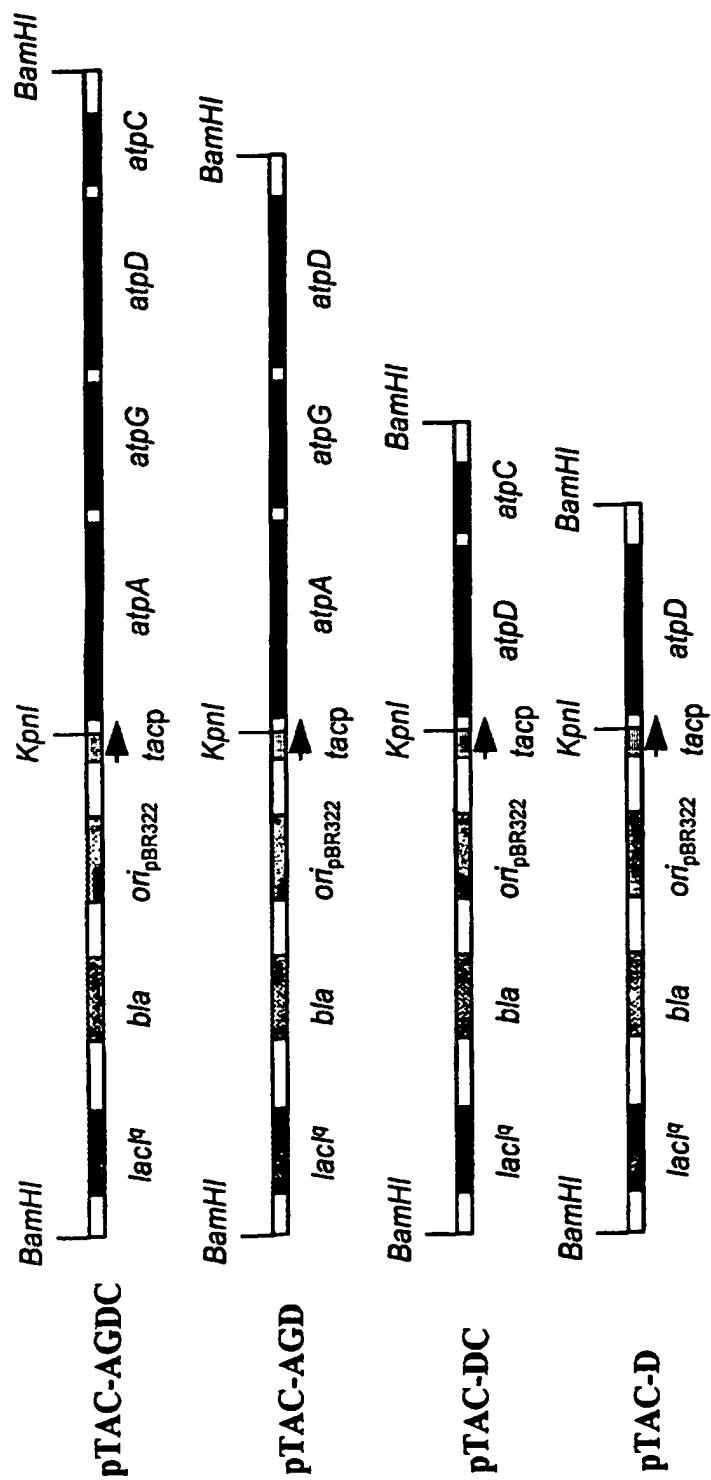
15

33. A method according to claim 32, wherein said DNA encoding F₁ or a portion thereof is derived from *Saccharomyces cerevisiae*, *Phaffia rhodozyma* or *Trichoderma reesei* and is selected from the group consisting of the gene encoding the F₁ subunit β or a portion thereof and various combinations of said gene or portion with the genes encoding the other F₁ subunits or portions thereof.

20

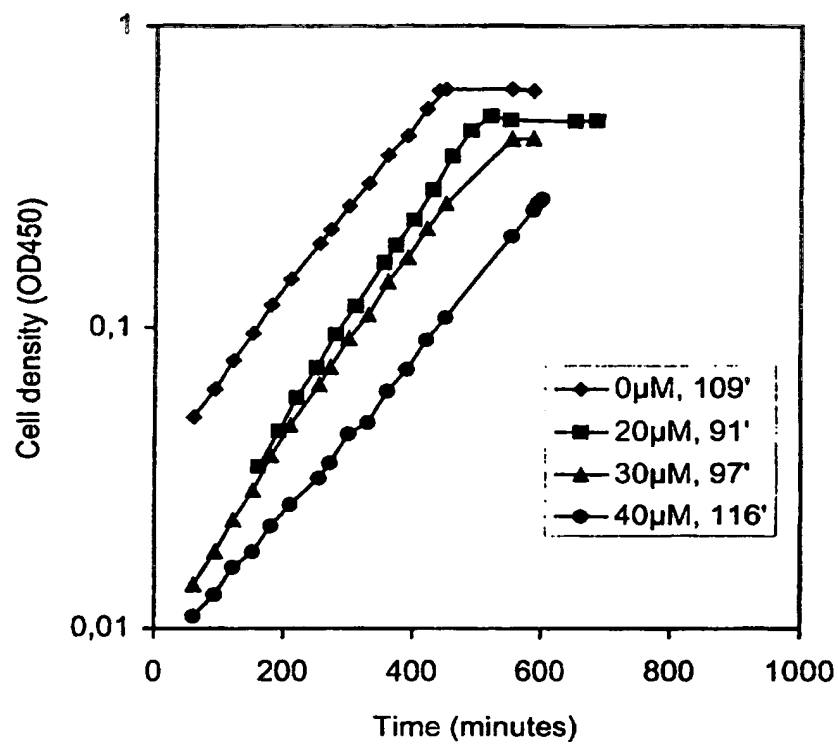
1/4

Fig.1



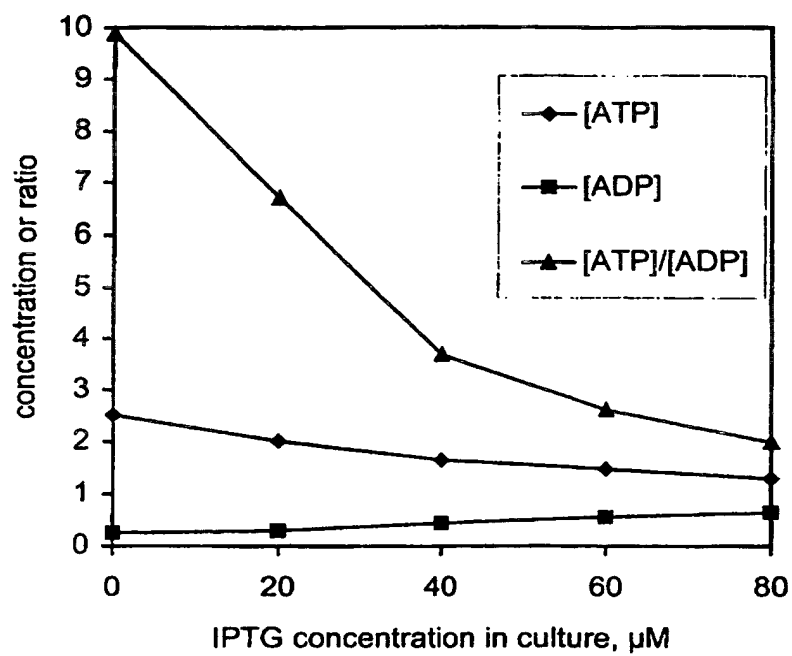
2/4

Fig. 2



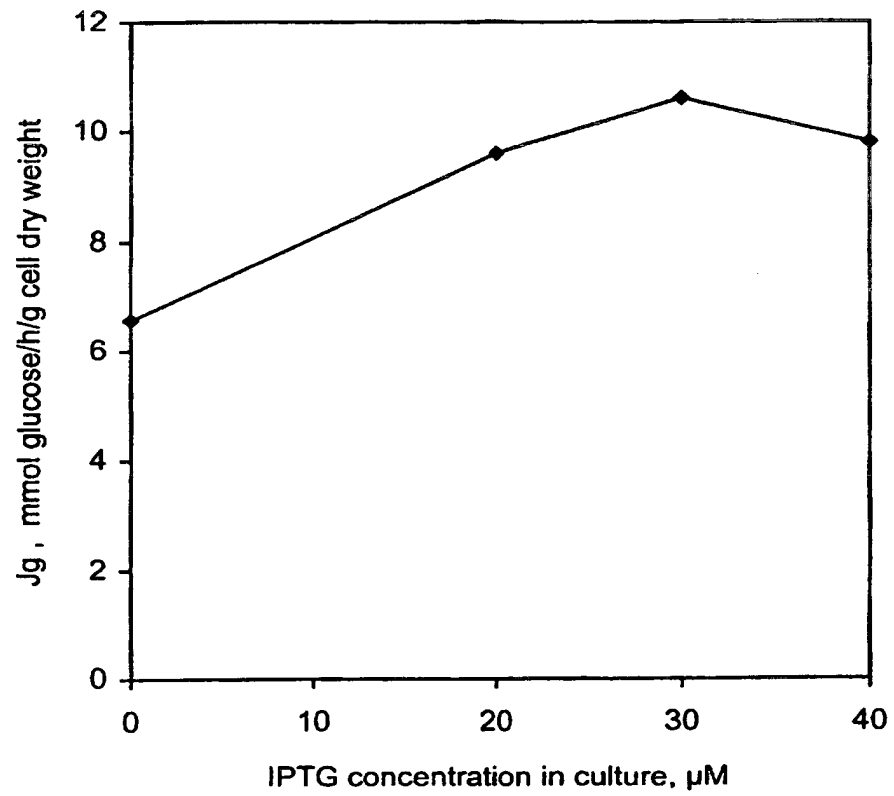
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Fig. 3



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Fig. 4



INTERNATIONAL SEARCH REPORT

International application No.

PCT/DK 97/00373

A. CLASSIFICATION OF SUBJECT MATTER

IPC6: C12P 1/00, C12N 15/67

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC6: C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

SE,DK,FI,NO classes as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

WPI, CA, MEDLINE, BIOSIS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 8703006 A1 (GENETICS INSTITUTE, INC.), 21 May 1987 (21.05.87), see page 7, line 3-11, page 25, line 5 - page 27, line 7	1,3-10,22
A	--	2,11-21, 23-33
A	EP 0645094 A1 (GIST-BROCADES N.V.), 29 March 1995 (29.03.95)	1-33
A	EP 0472286 A1 (MERCK & CO. INC.), 26 February 1992 (26.02.92), see claims	16-21

☒ Further documents are listed in the continuation of Box C.☒ See patent family annex.

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Date of the actual completion of the international search

4 December 1997

Date of mailing of the international search report

11-12-1997

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/DK 97/00373

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 9400493 A1 (KAPOOR, ARCHANA), 6 January 1994 (06.01.94), see claims -----	16-21

INTERNATIONAL SEARCH REPORT
Information on patent family members

01/10/97

International application No.
PCT/DK 97/00373

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